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L5	(laci or lipoprotein adj associated adj coagulation)	2648	L5
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☐ 54. 4863855 . 14 May 82; 05 Sep 89. Novel cloning vehicles for polypeptide expression in microbial hosts. Inouye; Masayori, et al. 435/69.1; 435/252.3 435/252.33 435/320.1 435/488 435/69.8 536/23.1 536/23.4 536/24.1 930/10 930/200. C12P021/00 C12N001/20 C12N015/00.

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Search Results - Record(s) 21 through 30 of 59 returned.

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☐ 21. 6307121 . 28 May 99; 23 Oct 01. Bacteriophage-based transgenic fish for mutation detection. Winn; Richard N.. 800/3; 536/23.1 536/23.7 800/20 800/21 800/25. G01N033/00 A01K067/027 C12N015/00 C07H021/02 C07H021/04.

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14/7/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

02121116 BIOSIS NO.: 000063036112
PENICILLIN INDUCED COAGULATION DISORDER
AUTHOR: ANDRASSY K; SCHERZ M; RITZ E; WALTER E; HASPER B; STORCH H; VOEMEL W
JOURNAL: LANCET 2 (7994). 1976 1039-1041. 1976
FULL JOURNAL NAME: Lancet
CODEN: LANCA
RECORD TYPE: Abstract

ABSTRACT: A coagulation disorder was seen after penicillin G administration (10 million units/day) in uremic patients and after high-dose penicillin G (40 million units/day) in patients with a normal glomerular filtration rate (5 patients after cardiac surgery). This disorder was characterized by prolongation of bleeding time, appearing immediately after penicillin G administration and persisting until 4 days after withdrawal of therapy; disturbance of collagen-induced and ristocetin-induced platelet aggregation; increase of antithrombin-III activity; and inhibition of factor-Xa activity. The inhibition of factor-Xa activity corresponded to that seen after low-dose heparin prophylaxis. The clinically latent coagulation disorder, when superimposed upon pre-existing coagulation abnormalities (uremia, treatment with anticoagulants) may cause severe bleeding, as observed in 1 patient with acute renal failure on hemodialysis.

14/7/2 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

03813081 EMBASE No: 1988262521
Factor VIII-bypassing activity of bovine tissue factor using the canine hemophilic model
O'Brien D.P.; Giles A.R.; Tate K.M.; Vehar G.A.
Department of Cardiovascular Research, Genetech, Inc., South San Francisco, CA 94080 United States
Journal of Clinical Investigation (J. CLIN. INVEST.) (United States) 1988, 82/1 (206-211)
CODEN: JCINA ISSN: 0021-9738
DOCUMENT TYPE: Journal
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

The bleeding disorder of hemophilia A currently treated by replacement therapy of the missing coagulation factor, factor

Discussion

These results show an inverse relationship between mean nitrite and hydrogen ion concentrations in the fasting gastric juice of all groups studied. The "normal" and duodenal-ulcer patients have similar hydrogen ion concentrations, and in both groups nitrite is present in very low concentrations of less than 2 μmol/l. The gastric cancer group have a low hydrogen ion concentration, as expected, and a much higher mean nitrite concentration of 38·8 μmol/l. The gastric-ulcer group occupy an intermediate position both in terms of hydrogen ion and nitrite concentrations. The higher nitrite concentrations are not due to the presence of gastric ulcer or cancer per se, since neither the nitrite nor hydrogen ion concentrations in these groups differed significantly from the group with fasting hypochlorhydria but no identifiable gastroduodenal lesion (the "hypochlorhydric normals"). This suggests that the high nitrite concentrations are related to the presence of fasting hypochlorhydria, irrespective of gastric lesion. The rate of N-nitrosation is determined by the concentration of nitrosatable amine and the square of the nitrite concentration.[14] Thus the higher nitrite concentration found in hypochlorhydric gastric juice may be sufficient to allow intragastric formation of carcinogenic nitrosamines. A nitrite concentration of 60 μmol/l has been shown to lead to significant nitrosamine formation in saliva[9] but lower concentrations have not, to our knowledge, been tested.

Thiocyanate is a powerful catalyst of nitrosation, and a thiocyanate concentration of 1 mmol/l will accelerate the in-vitro nitrosation of morpholine at an acid pH by 550 times;[13] its ability to participate in bacterial nitrosation is unknown. The mean gastric juice thiocyanate concentration in our patients was 1·5 mmol/l and the thiocyanate concentration of smokers was significantly greater than that of non-smokers, presumably reflecting salivary differences due to the absorption and metabolism of cyanide compounds from tobacco smoke.[13]

In the absence of bacteria the nitrosation reaction proceeds optimally at an acid pH.[14] However, nitrosation occurs readily at a neutral pH in the presence of many gastrointestinal bacteria.[15,16] In this study bacteria were recovered from nearly all samples of gastric juice, but both total bacterial counts and counts of nitrate-reductase-positive organisms were much higher in the patients with fasting hypochlorhydria, as defined by a hydrogen ion concentration of less than 5 mmol/l and pH greater than 5. The bacteria cultured from the acid specimens, with hydrogen ion concentrations greater than 15 mmol/l and pH less than 2·5, were presumably swallowed contaminants rather than established gastric residents and would be metabolically inactive at this pH. In contrast the bacterial flora of the neutral and near neutral stomach would be highly metabolically active, and capable of nitrate reduction, as bacterial nitrate-reductase activity is optimal at neutral pH.[17] The bacterial reduction of nitrate derived from saliva is the most likely source of the gastric nitrite, and this reduction may occur in the mouth[22] as well as in the neutral stomach. Nitrite entering the stomach in swallowed saliva would only persist at a high pH as nitrite is unstable in acid solution. In neutral gastric juice it would be supplemented by additional nitrite formed by intragastric bacterial reduction of residual salivary nitrate, and this is the probable explanation of the high nitrite concentrations found in our "hypochlorhydric" patients.

The presence of a high nitrite concentration together with a high bacterial count in the gastric juice of patients with fasting hypochlorhydria would be conducive to N-nitrosation. This would lead to the intragastric formation of carcinogenic nitrosamines and may be a factor in the known association between hypochlorhydria and gastric cancer.[1,23] The metabolically active bacteria present in neutral gastric juice could be important in this context, both by generating nitrite and catalysing nitrosasamine formation.

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Requests for reprints should be addressed to W. S. J. R.

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PENICILLIN-INDUCED COAGULATION DISORDER

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Summary A coagulation disorder was seen after penicillin-G administration (10 million units/day) in uræmic patients and after high-dose penicillin G (40 million units/day) in patients with a normal glomerular filtration-rate (5 patients after cardiac surgery). This disorder was characterised by: prolongation of bleeding-time, appearing immediately after penicillin-G administration and persisting until 4 days after

withdrawal of therapy; disturbance of collagen-induced and ristocetin-induced platelet aggregation; increase of antithrombin-III activity; and inhibition of factor-Xa activity. The inhibition of factor-xa activity corresponded to that seen after low-dose-heparin prophylaxis. The clinically latent coagulation disorder, when superimposed upon pre-existing coagulation abnormalities (uræmia, treatment with anti-coagulants) may cause severe bleeding, as observed in 1 patient with acute renal failure on hæmodialysis.

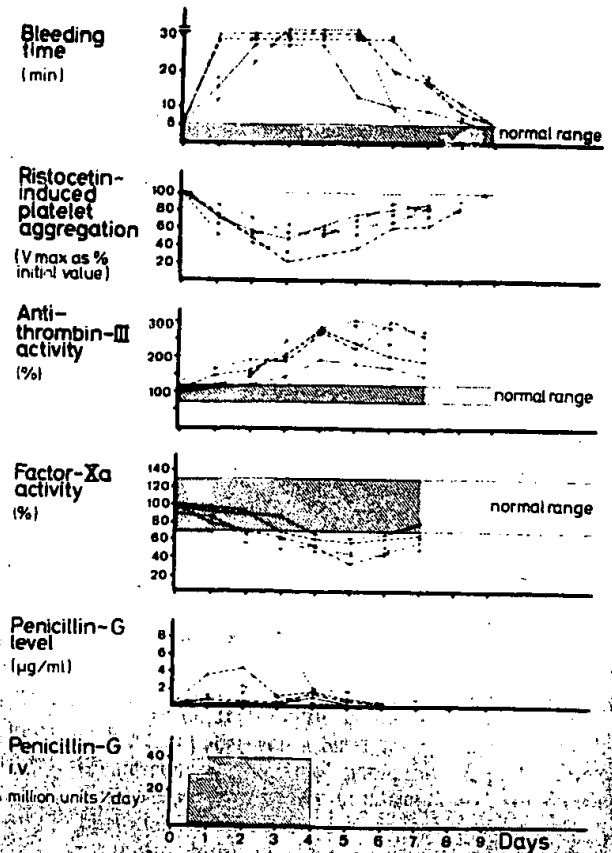
Introduction

APART from its well-known anaphylactic and immunological side-effects,[1-3] penicillin has remarkably low toxicity for man. When massive doses (e.g., 100 million units/day intravenously) are given,[4] central-nervous-system irritation with convulsions and coma may occur,[5 6] even in patients without renal failure. The same symptoms are seen with lower doses in patients with renal failure or when the blood-brain barrier is damaged (in cardiopulmonary bypass or meningitis).[4 7 8] However, apart from occasional observations of coagulation disturbances after topical application of penicillin[9] and reports of an acquired inhibitor of factor VIII,[10] coagulation disorders resulting from systemic administration of penicillin G have not been reported.

We have observed a hæmorrhagic diathesis with high serum-concentrations of penicillin G. High concentrations are sometimes seen in patients with renal failure or bacterial endocarditis. The coagulation disorder is the composite result of platelet dysfunction, disturbed conversion of fibrinogen to fibrin, and increased antithrombin-III activity.

Methods

Bleeding-time was measured by the method of Ivy et al.,[11] antithrombin III after Quick,[12] antithrombin-III protein with Mancini's technique using 'Partigen' plates of Behring Co., Marburg, Germany, factor-xa after Biggs,[13] factor-xa inhibitor after Denson and Bonnar,[14] thrombin-time after Quick,[15] and reptilase-time after Funk.[16] Staphylococcal clumping was tested after Hawiger et al.[17] Platelet aggregation was induced,[18] by ristocetin (1·5 mg/ml) and collagen (5 µg/ml, Hormon Chemie, München, Germany) and measured with the Heinrich-Roka-Aggregometer by V_{max} determination. Serum-penicillin levels were measured with a penicillin-G-sensitive strain of *Bacillus subtilis* after Bauers.[19]



Hæmostasis under postoperative penicillin-G prophylaxis.

Case-report

A 59-year old woman was admitted on Dec. 22, 1975, with acute renal failure. 3 wk before admission she had local anæsthesia of the jaw for tooth extraction. On Dec. 15, she was admitted to another hospital because of submandibular abscess. She was febrile (39°C) and complained of locked jaw; she was given cephalothin (12 g/day) and gentamicin (120 mg/day). The fever subsided 3 days later, and the patient remained afebrile. Insufficient parenteral fluid had been administered. On Dec. 22, she was in acute renal failure with

COAGULATION TESTS IN WOMAN WITH ACUTE RENAL FAILURE*

Date	Penicillin G (per day)	Antithrombin III		Factor xa (%)	Thrombin-time (s)	Reptilase-time (s)	Bleeding-time	Staphylococcus clumping-test
		Activity	Protein					
Normal range		70–130%	17–30 mg/dl	70–130%	18–25	16–20	<6 min	—¶
Dec. 21, 1975	10 million units	115	28	68	18	14·5	4 min 30 sec	—
Dec. 23	10 million units†	260	30·5	27	22·1	20	>30 min	—
Dec. 24‡	10 million units	360	35	20	22	21·2		—
Dec. 27	10 million units	410§	30·5	34	21·3	17·8	>30 min	—
Dec. 29	10 million units	290	30·5	39	22·1	18·3	..	—
Jan. 1, 1976	Propicillin 3·5 g	..	..	..	..	..	..	
Jan. 2	"	250	26	44	19·8	18·3	..	
Jan. 5	Propicillin 1·4 g	200	28	56	21·6	18·4	..	
Jan. 7	"	170	28	54	21·2	17·5	3 min 56 sec	
Jan. 9	"	155	27	60	21·6	16·4	..	

* See case-report.
† Measured serum-levels of penicillin G+=140 µg/ml.
‡ Blood-transfusion.
§After addition of protamine chloride (270 µg/ml)=115%.
¶—=No significant clumping, titre <2.

oliguria (200 ml/24 h) with high serum-urea (247 mg/dl, 41 mmol/l) and serum-creatinine (22 mg/dl, 1975 μmol/l). A fluctuating abscess in the submandibular region was palpable. Because mixed anaerobic infection was suspected, parenteral penicillin G, 10 million units/day, was administered. A Scribner shunt was inserted into the left forearm and hæmodialysis (6 h/day, average heparin dosage 6777±1481 I.U.) was begun immediately. There was considerable bleeding from the Scribner shunt cut-down. On Dec. 23, epistaxis began and continued for 2 days, necessitating nasal tamponade for 3 days (Blalock tamponade). Also on Dec. 23 the abscess was incised. This was followed by profuse hæmorrhage from the wound-bed, necessitating repeated tamponade. The estimated blood-loss was 1000 ml. Bleeding-time rose to >30 min and reverted to normal upon withdrawal of penicillin, although uræmia was still present and hæmodialysis was required. In parallel with the increase in bleeding-time, reptilase-time, though still within the normal range, was longer than the initial value. Thrombin-time was also slightly longer than initially. Antithrombin-III activity was increased and factor Xa reduced, presumably because of increased factor-Xa-inhibitor activity. The reduction of factor-Xa time was equivalent to a serum-heparin concentration of 0·13 units/ml (after Denson).[14] There was also a slight initial rise and subsequent fall in antithrombin-III-protein concentration. However, because of ultrafiltration and hypercatabolism, initial rise and later fall are difficult to interpret. Penicillin levels and coagulation studies before, during, and after therapy are summarised in the table.

Findings in Valvotomy Patients

5 patients (age 33±16 y, 1 male, 4 female) who had undergone mitral valvotomy were given 30 million units of penicillin G on the 1st postoperative day and 40 million units for another 3 days by intravenous infusion (2×20 million units/day as 1 h infusion). Serum-creatinine was 1·1 mg/dl (100 μmol/l) and creatinine clearance was >80 ml/min/1·73 m² in all patients. None of the patients received platelet-aggregation inhibitors, plasma expanders, or anticoagulants, nor were they on cardiac bypass. Penicillin levels were measured 11 h after the last penicillin infusion; at the same time bleeding-time was measured and platelet-counts, platelet-aggregation tests, and coagulation analysis were done (see figure). Mean platelet-counts were 184 800±29 400/mm³ initially and 198 800±36 465/mm³ at the end of the study. The difference was not significant. During the study platelet-counts, obtained daily, did not deviate more than 15% from the initial value. The measurements showed consistent prolongation of bleeding-time, increased antithrombin-III activity, and diminution of factor-Xa activity when compared with values before therapy. Bleeding-time rose within 24 h to values consistently above 20 min and did not return completely to normal until 4 days after withdrawal of penicillin. The changes in bleeding-time were paralleled by a reduction of collagen-induced and ristocetin-induced platelet aggregation (von Willebrand's disease was excluded in all patients by normal factor-VIII activity and normal platelet function before penicillin). Changes in the plasmatic system (antithrombin-III activity, factor-Xa activity) were slower to appear and slower to disappear than the platelet disorder. In addition to the values given in the figure, a slight prolongation of reptilase-time (from 20·0±0·8 s to peak value of 26·5±2·27 s on the 4th postoperative day) was observed; thrombin-time did not change; fibrin/fibrinogen degradation products were not raised (staphylococcus clumping-test). There was no consistent change of antithrombin-III protein concentration. The above changes were not seen in 5 patients with commissurotomy who received only 2×5 million units penicillin G/day. In vitro, the alteration of the plasmatic system could not be reproduced by addition of penicillin G to normal plasma (500 units penicillin G/ml).

Discussion

Bleeding is a well-known complication of carbenicillin therapy. The case described above shows that bleeding may also result from high-dose penicillin-G therapy. In previous studies, it was shown that penicillin derivatives, particularly carbenicillin,[20–23] inhibit platelet aggregation, interfere with the conversion of fibrinogen to fibrin, and increase antithrombin-III activity, possibly through release of heparin. The above findings are compatible with an analogous mechanism of penicillin G. The prolongation of bleeding-time after penicillin G points to disturbed platelet function, and the prolongation of reptilase-time is compatible with disturbed conversion of fibrinogen to fibrin, which was demonstrated by us using the Belitser assay system.[23] According to Biggs,[13] the decreased factor-Xa activity points to increased factor-Xa-inhibitor activity; this finding, as well as increased antithrombin-III activity, is compatible with the presence of heparinæmia, although direct evidence for this hypothesis is lacking. Absorption of increased antithrombin-III activity by $Al(OH)_3$ was poor, but addition of protamine chloride in vitro (270 μg/ml plasma) resulted in a striking reduction of antithrombin-III activity. It is not known whether administration of protamine chloride in these patients would also be of clinical benefit. The level of factor-Xa inhibition in the patients treated with high-dose penicillin corresponds to that seen after low-dose heparin prophylaxis.[24]

This study shows that high doses of penicillin cause a latent coagulation disorder which may become clinically manifest when it is superimposed upon pre-existing hæmostatic defects, as in uræmia or after surgery. It is possible that the hæmorrhagic diathesis of endocarditis lenta, which has never been satisfactorily explained, and the risk of bleeding after renal biopsy in patients with Löhlein's nephritis could stem from high-dose penicillin therapy. Many patients with endocarditis have reduced renal function (as a result of low cardiac output and/or concomitant glomerulonephritis) and are thus liable to have high penicillin levels.

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Requests for reprints should be sent to K. A., Medizinische Universitätsklinik, Bergheimerstrasse 58, 6900 Heidelberg, Federal German Republic.

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03640083 EMBASE No: 1988089519
The lipoprotein-associated coagulation inhibitor that inhibits the factor VII-tissue factor complex also inhibits factor Xa: Insight into its possible mechanism of action
Broze Jr. G.J.; Warren L.A.; Novotny W.F.; Higuchi D.A.; Girard J.J.; Miletich J.P.
Jewish Hospital, St Louis, MO 63110 United States
Blood (BLOOD) (United States) 1988, 71/2 (335-343)
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LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

Blood coagulation is initiated when plasma factor VII(a) binds to its essential cofactor tissue factor (TF) and proteolytically activates factors X and IX. Progressive inhibition of TF activity occurs upon its addition to plasma. This process is reversible and requires the presence of VII(a), catalytically active Xa, Casup 2sup +, and another component that appears to be associated with the lipoproteins in plasma, a lipoprotein -associated coagulation inhibitor (LACI). A protein, LACI(HG2), possessing the same inhibitory properties as LACI, has recently been isolated from the conditioned media of cultured human liver cells (HepG2). Rabbit antisera raised against a synthetic peptide based on the N-terminal sequence of LACI(HG2) and purified IgG from a rabbit immunized with intact LACI(HG2) inhibit the LACI activity in human serum. In a reaction mixture containing VIIa, Xa, Casup 2sup +, and purified LACI(HG2), the apparent half-life (t1/2) for TF activity was 20 seconds. The presence of heparin accelerated the initial rate of inhibition threefold. Antithrombin IIIalpha alone had no effect, but antithrombin IIIalpha with heparin abrogated the TF inhibition. LACI(HG2) also inhibited Xa with an apparent t1/2 of 50 seconds. Heparin enhanced the rate of Xa inhibition 2.5-fold, whereas phospholipids and Casup 2sup + slowed the reaction 2.5-fold. Xa inhibition was demonstrable with both chromogenic substrate (S-2222) and bioassays, but no complex between Xa and LACI(HG2) could be visualized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Nondenaturing PAGE, however, showed that LACI(HG2) bound to Xa but not to X or Xa inactivated by diisopropyl fluorophosphate. Thus, LACI (HG2) appears to bind to Xa at or near its active site. Bovine factor Xa lacking its gamma-carboxyglutamic acid-containing domain, BXa(-GD), through treatment with alpha-chymotrypsin, was used to further investigate the Xa requirement for VIIa/TF inhibition by LACI(HG2). LACI(HG2) bound to BXa(-GD) and inhibited its catalytic activity against a small molecular substrate (Spectrozyme Xa), though at a rate approximately sevenfold slower than native BXa. Preincubation of LACI(HG2) with saturating concentrations of BXa(-GD) markedly retarded the subsequent inhibition of BXa. The VII(a)/TF complex was not inhibited by LACI (HG2) in the presence of BXa(-GD), and further, preincubation of LACI

Kelly J P; Thomas L; Moulder P V; Webb W R
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Main Citation Owner: NLM
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Clinical and laboratory experience with circulating lupus anticoagulant in 3 patients undergoing coronary artery bypass procedures is reported. This circulatory anticoagulant inhibits activation of prothrombin by the prothrombin activator complex (factor Xa, factor V, and phospholipid). The presence of lupus anticoagulant was initially detected because of a prolonged activated partial thromboplastin time and a normal or mildly prolonged prothrombin time. The 3 patients underwent uncomplicated coronary artery bypass grafting and experienced no abnormal bleeding postoperatively. The lupus anticoagulant is a rare cause of bleeding after open-heart surgery. It appears to be a problem only when an additional coagulation defect is present.
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14/7/5 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

04938601 85245383 PMID: 4012669
Comparative effects of heparin and PK 10169, a low molecular weight fraction, in a canine model of arterial thrombosis..
Mestre M; Clairefond P; Mardiguian J; Trillou M; Le Fur G; Uzan A
Thrombosis research (UNITED STATES) May 15 1985, 38 (4) p389-99, ISSN 0049-3848 Journal Code: 0326377
Document type: Journal Article
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Record type: Completed
The comparative properties of heparin and PK 10169, a low molecular weight fraction, were studied using an antithrombotic test in anaesthetized dogs. The antithrombotic properties of the two compounds were evaluated by measuring inhibition of thrombus formation following transluminar stimulation of coronary artery with anodal current and by measuring anticoagulant properties, anti Xa and anti IIa activities. The results show that PK 10169 displayed significant antithrombotic activities above 0.625 mg/kg and was equipotent at 2.5 mg/kg s.c. with heparin 10 mg/kg s.c. No correlation could be observed between antithrombotic/anti Xa ratio of both compounds. Moreover it was shown that, unlike heparin, PK 10169 s.c. was devoid of obvious anticoagulant properties and induced a negligible anti IIa activity contrasting with a high anti Xa level. A similar dissociation between anti Xa and anti IIa activities was observed following i.v. administration of 2.5 mg/kg of PK 10169 but not with heparin. This low molecular weight heparin fraction might thus be regarded as a potential arterial antithrombotic agent devoid of appreciable anticoagulant effect.
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14/7/7 (Item 1 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

104202975 CA: 104(23)202975y JOURNAL
Isolation and characterization of human antithrombin III
AUTHOR(S): Trobisch, H.; Wuest, T.; Rustige, H.

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THROMBOSIS RESEARCH 38; 389-399, 1985
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COMPARATIVE EFFECTS OF HEPARIN AND PK 10169, A LOW MOLECULAR WEIGHT FRACTION, IN A CANINE MODEL OF ARTERIAL THROMBOSIS

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ABSTRACT

The comparative properties of heparin and PK 10169, a low molecular weight fraction, were studied using an antithrombotic test in anaesthetized dogs. The antithrombotic properties of the two compounds were evaluated by measuring inhibition of thrombus formation following transluminar stimulation of coronary artery with anodal current and by measuring anticoagulant properties, anti Xa and anti IIa activities. The results show that PK 10169 displayed significant antithrombotic activities above 0.625 mg/kg and was equipotent at 2.5 mg/kg s.c. with heparin 10 mg/kg s.c. No correlation could be observed between antithrombotic/anti Xa ratio of both compounds. Moreover it was shown that, unlike heparin, PK 10169 s.c. was devoid of obvious anticoagulant properties and induced a negligible anti IIa activity contrasting with a high anti Xa level. A similar dissociation between anti Xa and anti IIa activities was observed following i.v. administration of 2.5 mg/kg of PK 10169 but not with heparin. This low molecular weight heparin fraction might thus be regarded as a potential arterial antithrombotic agent devoid of appreciable anticoagulant effect.

INTRODUCTION

Heparin is considered as the drug of choice in the treatment of acute venous thromboembolic disease. The therapeutic effects of heparin are mediated through activation of antithrombin III, making it a potent coagulation inhibitor. However, the haemorrhagic effects of this compound are a major drawback to its use, which has stimulated much effort aimed at producing safer derivativ s. Recently, polysaccharide fractions of lower molecular weight (LMWH) have been prepared. Unlike h parin these produc a relatively greater inhibition of the activated Xa clotting time than of the thrombin time or the activated partial thromboplastin time. In vivo, these

Key words: Low molecular weight heparin, PK 10169, heparin, antithrombotic properties

compounds produced a significant antithrombotic effect in experimental models of venous thrombosis (induced in animals by combination of stasis and injection of a thrombogenic mixture). However, the efficacy of LMWH in arterial thrombosis has not previously been demonstrated clearly. As the aetiology and pathogenesis of arterial thrombosis in man is still obscure, justification of an experimental model in animals can nly be, at best, circumstantiel. However models of coronary thrombosis, which is associated dramatically with transmural myocardial infarction, must fulfil at least two criteria : it should have a good analogy with human thrombosis, and produce a disturbance of coronary blood flow as a consequence and not a cause of the thrombosis. Salazar (1) proposed in 1961 that an arterial coronary thrombosis could be induced by application of anodal current to the intravascular lumen, via a stainless steel electrode, positioned under fluoroscopic control. In 1981 Romson et al. (2) demonstrated a simpler technique which avoided the need for fluoroscopy. They showed that their experimental intravascular thrombus was morphologically similar to that of naturally occurring thrombi.

The availability in our laboratory of a well defined LMWH, PK10169, prompted us to investigate its effect on this experimental model of arterial coronary thrombosis. PK 10169, prepared from porcin heparin by using a selective and well controlled depolymerization procedure, has a molecular weight 3 to 4 times lower than heparin and a higher anti Xa / anti IIa ratio (3). Our study has investigated blood coagulation, haemodynamic parameters and thrombus formation following subcutaneous or intravenous administration of heparin or PK 10169 to anaesthetized dogs.

METHODS

70 adult mongrel dogs of either sex (16 to 25 kg) were preanaesthetized with thiopental sodium (500 mg/kg I.V.) and maintained under anaesthesia with pentobarbital sodium as required. Artificial respiration was established with a Bird mk2 respirator. Blood gas were measured and maintained to standard values (pH = 7.35 $\pm$ 0.2 ; pO_2 = 100 $\pm$ 5 mm Hg ; pCO_2 = 40 $\pm$ 5 mm Hg). The femoral artery and saphenous vein were cannulated to allow blood pressure recording, blood sampling and drug injection. A thoracotomy was made through the 4th left intercostal space, the pericardium was opened and the main circumflex artery was carefully dissected 2 cm below its origin. Coronary blood flow was monitored with an electromagnetic flow probe (Nycotron 376). A platinum wire electrode, insulated with polyethylene, was secured to the myocardium with the tip positioned inside the artery. An anodal current (150 A) was delivered between this electrode and subcutaneous steel hypodermic needle on the 7th rib, according to Romson et al (2). The surface ECG lead II was monitored and the heart rate was trigged from the QRS complex. Haemodynamic and electrocardiographic parameters were recorded on a 8 channel HP 7758B polygraph system. The preparation was maintained stable for 30 minutes before delivery of any current or compounds. Electrical stimulation was maintained continuously over 6 hours and dogs killed with KCl. The heart was removed and thrombi weighed and photographed after arteriotomy. Control blood samples were obtained at T -30, TO and at hourly intervals for measurement of bl od heparin levels. Blood clotting time (BCT) and plasma recalcification time (PRT) were measured by standard procedures. Anti Xa and anti IIa activi-

ti s were measured spectrometrically in the plasma using chromogenic substrates (Automated Gilford 2035). Standard curves were obtained with addition of increasing quantities f PK 10169 and heparin in plasma. Biological activities of the two compounds were calculated from following indexes of activities :

	Mean molecular weight	Anti Xa U/mg	Anti IIa U/mg	$\frac{\text{Anti Xa}}{\text{Anti IIa}}$ ratio
Standard heparin (FF 10238)	16,000	137	132	1
PK 10169 (FF 931)	4,500	112	28	4

Subcutaneous injection of either PK 10169 or heparin in a volume of 5 ml were made into the 6th intercostal space. Intravenous administration involved a bolus infusion of 1.25 mg/kg followed by perfusion of 1.25 mg/kg for 6 hours. This was necessary on account of the short lasting plasma concentration of these compounds.

RESULTS

1. Thrombus development

Transluminar coronary stimulation in dogs induced a time dependent thrombus development over a period of 6 hours. The weights of coronary thrombi increased from 7.7 ± 1.25 mg (n = 3) to 24.8 ± 2.2 mg (n = 11) between 2 and 6 hours of electrical stimulation .espectively (fig. 1). These data agree with those of Schumacher et al (4). There was no correlation between weight and length of thrombi. Progressive coronary occlusion over 6 hours reduced coronary blood flow by more than 50 % in 9 out of 11 animals (with a mean overall of 64.7 %). Blood pressure and heart rate remained unchanged during the experiment. Neither BCT nor PRT changed significantly over the 6 hours of experiments ; their values were 3.17 ± 0.18 and 2.90 ± 0.17 min respectively. There was no detectable anti Xa or anti IIa activity at any time during the experiment.

2. Antithrombotic effect of PK 10169 and heparin

Subcutaneous administration of either PK 10169 or heparin had antithrombotic effects but at different doses : PK 10169 was very effective from 0.625 mg/kg ($p < 0.05$) whereas doses of heparin of over 2.5 mg/kg were required (fig. 2). The effect of PK 10169 reached a plateau at doses

high r than 2.5 mg/kg (thrombus weight : 9.05 ± 0.85 mg) whilst heparin did not achieve an equivalent effect with 20 mg/kg (thrombus weight : 7.11 ± 0.93 mg). Th thrombi obtained with the highest doses of th two compounds (i.e. 5-9 mg) did not impede coronary blood flow.

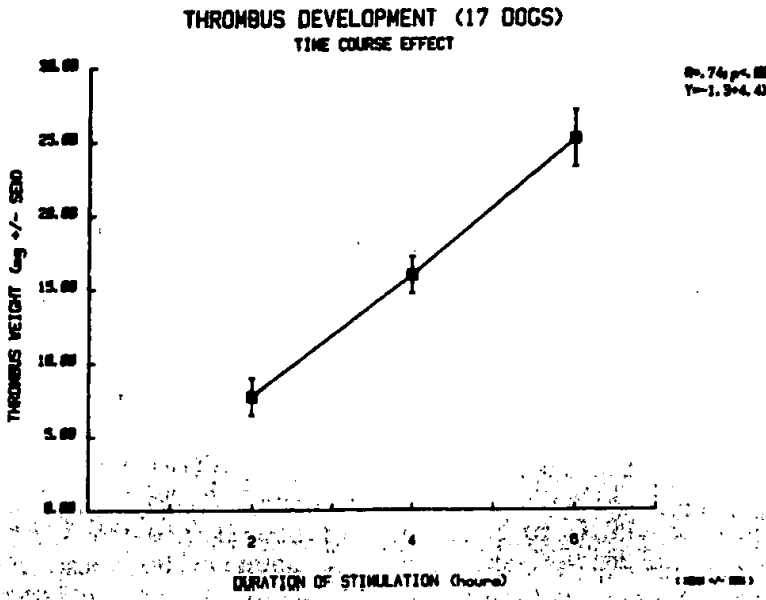

FIG. 1

Time course of development of coronary thrombi in untreated anaesthetized dogs (n = 20). Thrombus weight is expressed in mg ± SEM and duration of electrical stimulation in hours. The thrombus present at the site of electrical injury was removed after 2, 4 and 6 hours of stimulation and immediately weighed on an analytical balance. Time dependency was calculated by linear regression analysis ($r = 0.74$; $p < 0.01$; $y = -1.3 + 4.4x$).

The thrombi obtained with the highest doses of the two compounds (i.e. 5-9 mg) did not impede coronary blood flow. In that way reduction in coronary flow observed in the control group (- 64.73 ± 8 %) was significantly altered by PK 10169 (fig. 3) at a dose of 1.25 mg/kg s.c. (- 22.03 ± 13.68 ; $p < 0.01$). In spite of intragroup variations with the two highest doses this effect was dose-dependent and correlated with thrombus weight (fig. 4). On the other hand, heparin induced a significant ($p < 0.05$) but not clearly dose-dependent inhibition of restriction of coronary flow. This difference is related to greater interindividual variations. Neither PK 10169 nor h parin altered arterial blood pressur or heart rate throughout the experiment.

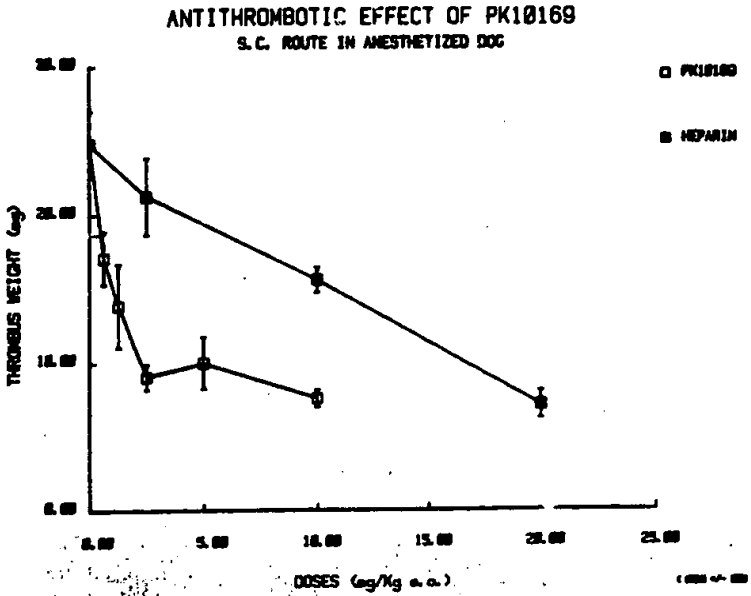

FIG. 2

Antithrombotic effect of increasing doses of PK 10169 and heparin (S.C.) in anaesthetized dogs. The two compounds were injected 30 min before the onset of stimulation (total duration of 6 hours). After sacrifice thrombi were weighed as described previously. Each treated group represents 5 dogs. PK 10169 was administred at 0.625, 1.25, 2.5, 5 and 10 mg/kg S.C. and heparin at 2.5, 10 and 20 mg/kg s.c. The control group represents 11 dogs. Each point represents the mean ± SEM and significance calculated from residual analysis of variance using a t'test as modified by Dunnett (5). Correlations were obtained from regression analysis pack (HP 85).

PK 10169 : $r = 0.54$; $p < 0.01$; $y = 46.9 - 4.8\,x$
Heparin : $r = 0.6$; $p < 0.01$; $y = 0.2 + 2.2\,x$

PK 10169 induced a slight increase in BCT and PRET (fig. 5) which increased respectively from control values of 3.18 ± 0.18 min and 2.90 ± 0.18 min to 3.57 ± 0.25 min and 3.86 ± 0.36 min with 2.5 mg/kg s.c. and to 6.58 ± 0.63 min and 6.79 ± 1.13 min with the highest dose of PK 10169 (10 mg/kg). In contrast, heparin largely and dose-dependently increased these two factors from about 3 to 20 minutes in the same conditions. This increase could be correlated with variations in anti IIa activity ($r = 0.98$; $p < 0.01$). PK 10169 is however devoid f such activity even at the highest doses.

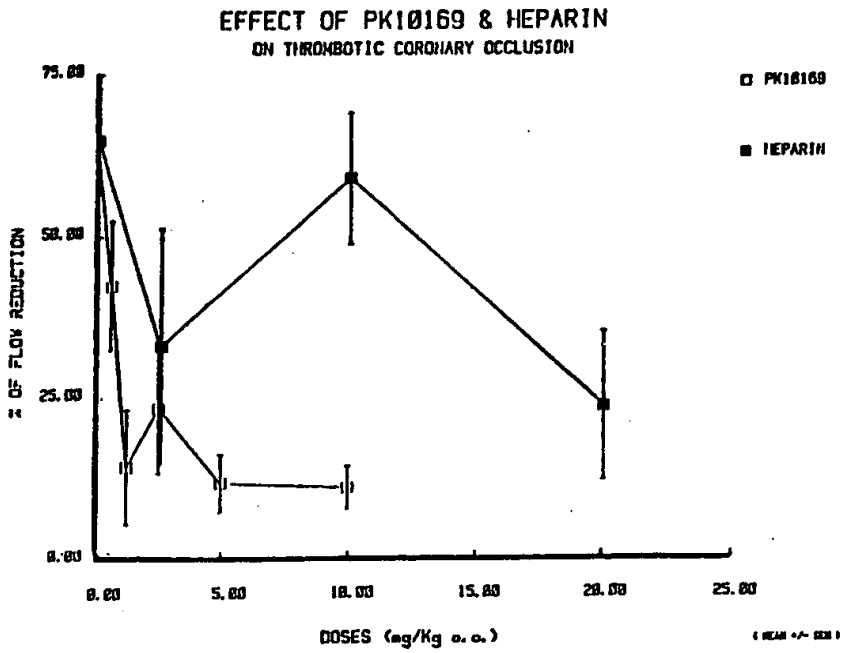

FIG. 3

Effect of PK 10169 and heparin on thrombotic coronary occlusion in the anaesthetized dog. PK 10169 and heparin were administred by the s.c. route as described in fig. 3 and "Methods"". The effect of each compound is expressed as the mean ± SEM in percent of flow reduction.

Dose vs thrombus weight :

PK 10169 : $r = 0.63$; $p < 0.01$; $y = 19.68 - 1.53\,x$
Heparin : $r = 0.63$; $p < 0.01$; $y = 24.33 - 0.87\,x$

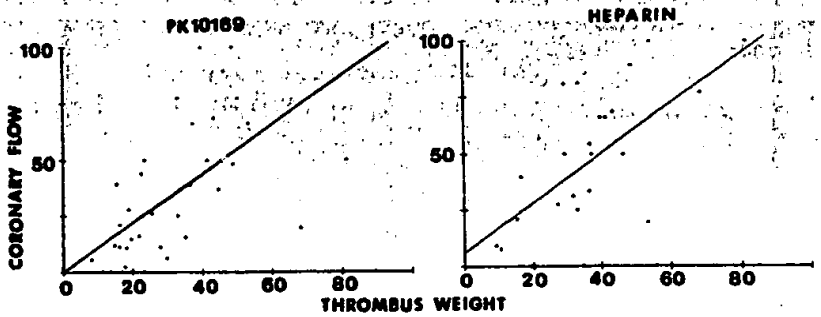

FIG. 4

Correlation between coronary flow and thrombus weight formation after 6 hours of stimulation following PK 10169 and heparin treatment. Each point represents the value of coronary flow (% flow reduction) for corresponding thrombus weight (mg) in each dog. Data were c ll ct d from 52 dogs. Th line was fit by mean of linear regr ssion analysis.

PK 10169 : $r = 0.599$; $p < 0.01$; $y = 0.23 + 2.17\,x$
Heparin : $r = 0.58$; $p < 0.01$; $y = 5.97 + 2.2\,x$

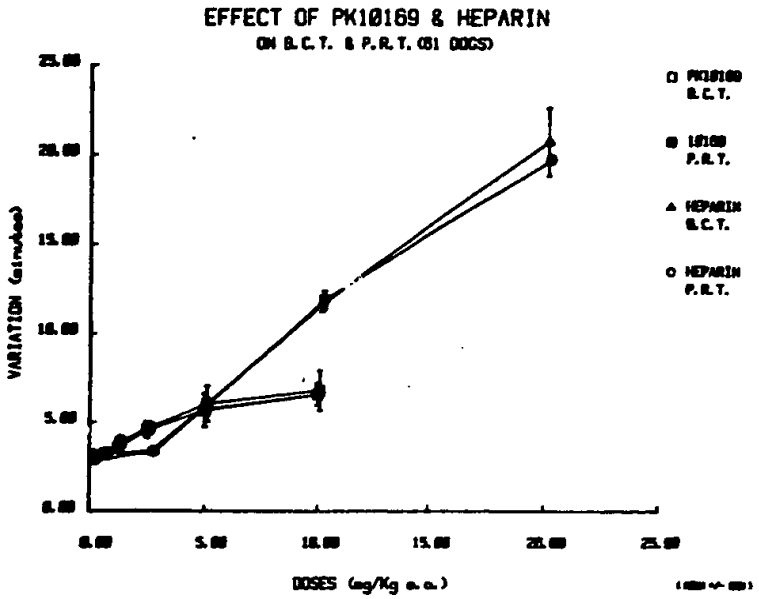

FIG. 5

Comparative effect of PK 10169 and heparin on blood coagulation time (BCT) and plasma recalcification time (PRT) in anaesthetized dogs. PK 10169 and heparin were administered by the s.c. route as described in fig. 2 and "Methods". The effect of each compound is expressed in minutes and each point as the mean ± SEM.

Heparin : BCT vs dose : $r = 0.89$; $p < 0.01$; $y = 1.06 + 0.01\ x$
BCT vs anti IIa activity : $r = 0.98$; $p < 0.01$; $y = -0.46 + 0.79\ x$

Both PK 10169 and heparin promote by the s.c. route a significant and dose-dependent anti Xa activity (fig. 6). The effect of 10 mg of heparin was obtained with half the dose of PK 10169 (5 mg/kg). By i.v. route, the short plasma life of heparin and the long duration of electrical stimulation (6 hours) necessitated slow perfusion of the compound over 6 hours following the initial bolus injection in order to maintain a sufficient plasma concentration. Under these conditions, PK 10169 and heparin induced a large and significant ($p < 0.01$) inhibition of thrombus development with 2.5 mg/kg i.v. It should be emphasised that, although BCT and PRT are dramatically increased by heparin, the effect of PK 10169 on these parameters were only minor albeit significant. The clear dissociation between anti Xa and anti IIa activities of PK 10169 i.v. was not observed with heparin (PK 10169 anti Xa activity was 3 to 4 times higher than anti IIa activity ; fig. 7). Moreover the anti Xa action of PK 10169 i.v. was about 2 times higher than that of heparin.

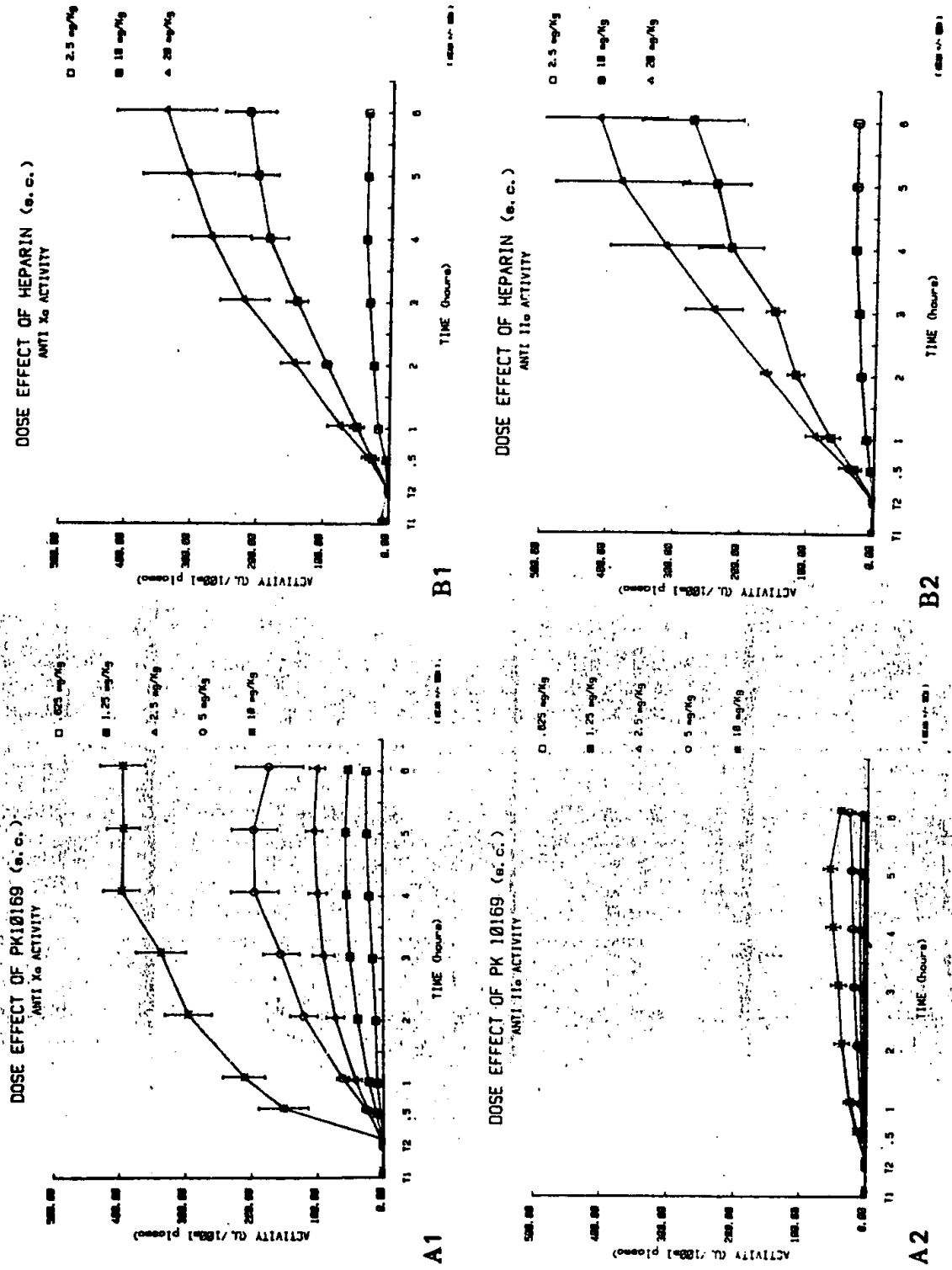

FIG. 6

Effect of PK 10169 (A) and heparin (B) on anti Xa (1) and anti IIa (2) activities in the anaesthetized dog. PK 10169 and heparin were administered by the s.c. route as previously described in fig. 3 and "Methods". The effect of each compound is expressed as unit/100 ml plasma. Each point corresponds to mean ± SEM.

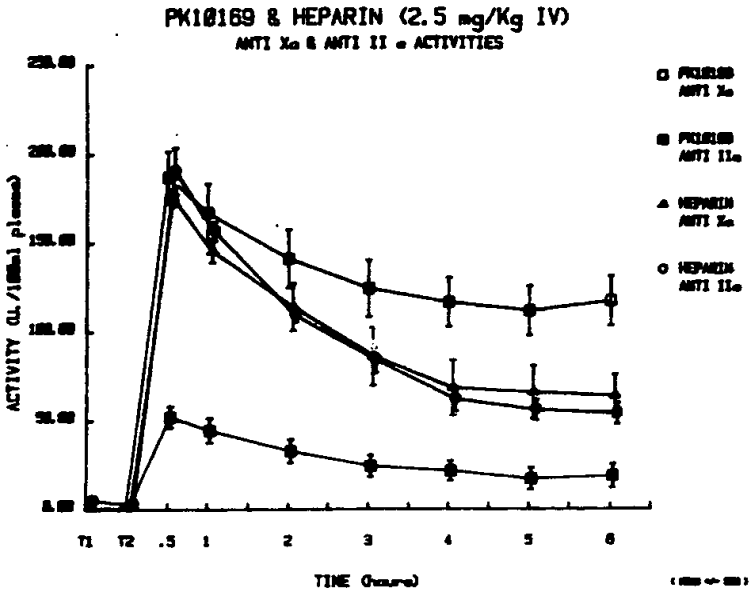

FIG. 7

Effect of PK 10169 and heparin on anti Xa and anti IIa activities in the anaesthetized dog. The compounds were administered i.v. at 1.25 mg/kg in bolus followed by 1.25 mg/kg in slow rate perfusion over 6 hours, as described in "Methods". The effect of each compound is expressed in unit/100 ml plasma. Each point corresponds to the mean ± SEM.

DISCUSSION

To our knowledge heparin and low molecular weight fractions have not yet been tested on an in vivo canine model of coronary artery thrombosis. This animal model, which does not involve vessel stasis or blood hypercoagulability, produces a thrombus similar in morphology and composition to human coronary artery thrombi (2). The low amperage electrical stimulation of the LCX induces platelet adhesion and aggregation followed by an incorporation of fibrin stabilizing the initial thrombus. Using the diminution of coronary blood flow during the course of the experiment as an index of thrombotic occlusion, Romson et al. (2) found that the average time for complete occlusion was 3.2 ± 0.4 hours of electrical stimulation and that after 6 hours, the mean thrombus weight was 50 ± 8 mg. In contrast, our results show a slower decrease of coronary blood flow, reaching only 74.73 ± 8.2 % of reduction of control values after 6 hours, related to a mean thrombus weight of 24.79 ± 2.2 mg. However, further studies from Schumacher et al. (4) show a final thrombus development similar to our data (23 ± 2 mg).

The antithrombotic effect of PK 10169 in the canine model of arterial thrombosis is higher than that of heparin after subcutaneous administration. ED 50 calculated from reductions in thrombus weight are respectively

about 0.77 mg/kg for PK 10169 and 10.66 mg/kg for heparin. This difference cannot be related to higher blood hypocoagulability since 0.625 mg/kg of PK 10169 did not alter BCT and PRT, whilst an equipotent dose of heparin increases these clotting times about 4 times. Furthermore, the anti IIa activity induced by 0.625 mg/kg f PK 10169 corresponds t 2.5 U/100 ml plasma whereas that induced by 10 mg/kg of heparin corresponds to 278 U/100 ml plasma. So hypocoagulability may not be considered as a parameter of inhibition of thrombus development in this model of arterial coronary thrombosis.

Surprisingly, the antithrombotic potency does not seem to be related to anti Xa activity : a similar reduction of thrombus weight was obtained after respective s.c. administration of 1.25 mg/kg and 10 mg/kg of PK 10169 and heparin, whereas anti Xa activities are 55.4 $\pm$ 4 U/100 ml plasma for PK 10169 and 216 $\pm$ 382 U/100 ml plasma for heparin. The efficacy of PK 10169 might be due to a better bioavailability after s.c. injection. Although PK 10169 and heparin have the same anti Xa activity in vitro and immediately after i.v. injection, s.c. kinetic curves are markedly different : PK 10169 reached a plateau at 4 hours whereas heparin did not, even at 6 hours. Moreover, the constant perfusion of 1.25 mg/kg of each compound for 6 hours, immediately following bolus injection, demonstrated as initially equipotent on anti Xa activity, points out a more rapid decrease after heparin than after PK 10169 infusion. This suggests a longer duration of action of PK 10169 although the final effect of both compounds, after i.v. administration on thrombus weight was similar.

Therefore, anti Xa activity might not be the only factor involved in the antithrombotic efficacy of PK 10169. Recently Hoppensteadt et al. (6) suggested that anti Xa and anti IIa activities cannot explain the overall antithrombotic action of heparin and derivatives and that other properties, such as interactions with contact system, fibrinolytic system, endothelium, platelets or prostacyclin/thromboxan systems might contribute to the antithrombotic response.

Besides the prevention of thrombus growth, PK 10169 partially prevents the reduction in coronary blood flow. However in control groups as well as in treated animals, coronary blood flow values are very variable. These variations might be due to anatomic anastomosis distal to the probe, adaptative reflexes to ischemia, variations in heart rate during ischemia, or to individual variations of arterial diameters. Our data show that LMWH PK 10169 possesses an in vivo good dissociation between antithrombotic activity and global blood coagulability, two properties which cannot be differentiated by heparin. Finally the biological parameters measured during our experiments are not directly predictive of the antithrombotic activity of PK 10169. This compound might be used therapeutically for preventing arterial thrombosis or reducing the extend of ischemic injury. Nevertheless measurement of anti Xa activity might be used as an indirect parameter for measuring plasma levels.

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Möglichkeiten der Isolierung und Charakterisierung des menschlichen Antithrombin III

Isolation and Characterization of Human Antithrombin III

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Summary:

Antithrombin III, the most important inhibitor of activated coagulation factors (II_a, IX_a, X_a, XI_a, XII_a) was isolated from human plasma by affinity chromatography. The biochemical characterization of this highly purified inhibitor showed, that is was uniform by means of HPLC and PAA gradient gel electrophoresis. The molecular weight was determined to 70000 daltons and the isoelectric point was pH 4,5.

The inhibition of factor X_a by AT III had been proofed as competitive by kinetic studies. AT III works as a "dead end inhibitor". Heparin seems to form a ternary complex with AT III and activated coagulation factors, so acting as a catalyst.

The therapeutic value of AT III-concentrates will be discussed.

Zusammenfassung

Antithrombin III, der wichtigste Inhibitor der aktivierten Gerinnungsfaktoren II, IX, X, XI und XII, wurde mit Hilfe der Affinitätschromatographie aus menschlichem Plasma isoliert. Weitere Untersuchungen durch den Einsatz der HPLC und der PAA-Gradientengelelektrophorese zeigten die hohe Reinheit des Eluates. Das Molekulargewicht des so isolierten Antithrombin III betrug 70000 Daltons, der isoelektrische Punkt lag bei pH 4,5.

Durch Einsatz kinetischer Untersuchungsmethoden konnte gezeigt werden, daß die Hemmung von $F X_a$ durch das hochreine Antithrombin III einem kompetitiven Prinzip folgt. Heparin bildet einen ternären Komplex mit AT III und aktivierten Gerinnungsfaktoren und wirkt als Katalysator der AT III vermittelten Inhibition.

Möglichkeiten zum therapeutischen Einsatz von AT III-Konzentraten werden diskutiert.

Key Words: Historical review, antithrombin III-concentrate, heparin, activated serin-proteases, isolation of AT III by affinity chromatography, kinetic characterization, therapeutic aspects.

Einleitung

Entdeckung, Biochemie und Physiologie des AT III

Während die Entdeckung des Heparins bereits um die Jahrhundertwende durch mehrere Arbeitsgruppen teilweise in Unkenntnis voneinander[29,13,14,11,21,18] erfolgte, beschrieben erst *Brinkhouse* et al. im Jahre 1939[7] einen weiteren Plasmafaktor, den Heparin zur Hemmung der Prothrombinaktivierung benötigt. Ohne Heparin zeigte dieses, zu dieser Zeit noch nicht näher charakterisierte Protein, eine nur geringe Antithrombinwirkung.

1955 erfolgte durch *Monkhouse* et al.[23] eine detaillierte Spezifizierung der Antithrombine.

1. Antithrombin I bezeichnet die Absortion von kleineren Mengen Thrombin an das Fibringerinnsel.
2. Unter Antithrombin II versteht man den zur Heparinwirkung erforderlichen Plasma-Co-Faktor.
3. Antithrombin III bezieht sich auf die progressive Inaktivierung von Thrombin durch ein Plasmaprotein.
4. Antithrombin IV beschreibt einen nicht näher geklärten Effekt der Vernichtung von Thrombin sofort im Anschluß an seine Bildung aus Prothrombin.
5. Antithrombin V ist die Bezeichnung für ein Immunglobulin, das die Aggregation von Fibrinmonomeren verhindert.
6. Antithrombin VI ist identisch mit den hochmolekularen Fibrinspaltprodukten.

Gleichzeitig konnte gezeigt werden, daß die Funktion von Antithrombin II und Antithrombin III durch das gleiche Molekül erfüllt wird. In der internationalen Literatur spricht man nur noch von Antithrombin III, worunter sowohl der Effekt der progressiven Inhibition als auch die Heparin-Co-Faktor-Wirkung subsummiert werden.

Antithrombin III ist ein Glykoprotein, das aus einer einzigen Polypeptidkette besteht und eine molekulare Masse von 64.500 Dalton aufweist.

Das Protein liegt in einer Konzentration von etwa 15 mg/dl im Plasma vor und entspricht in seiner elektrophoretischen Beweglichkeit den Eiweißen der $alpha_2$-Globulinfraktion.

Antithrombin III wird in den Hepatozyten gebildet, wobei die Synthese im Gegensatz zu der der Gerinnungsfaktoren II, VII, IX und X sowie des Inhibitors Protein C unabhängig von der Vitamin K-Konzentration in der Leberzelle ist.

Die katabole biologische Halbwertszeit beträgt im gesunden Organismus ungefähr vier Tage. Im Falle einer Aktivierung des Gerinnungssystems etwa im Rahmen einer disseminierten intravasalen Koagulopathie (DIC) kann dieser Wert allerdings auf wenige Stunden absinken.

Die Funktion des Antithrombin III besteht darin, einen unter physiologischen Bedingungen nicht dissoziierenden Komplex mit Serinproteasen zu bilden. Zwar ist dies bisher vor allem am Beispiel des Thrombins gezeigt worden[27], jedoch ist diese Fähigkeit nicht auf das Thrombin beschränkt. Es können auch die aktivierten Gerinnungsfaktoren IX, X, XI und XII sowie das Plasmin gleichartig gehemmt werden[10,17,37,25,31,29]. Diese Inhibition kann durch Heparin im Sinne eines biologischen Katalysators maximal beschleunigt werden. Dazu wird Heparin an spezielle Bindungsstellen im Antithrombin III-Molekül fixiert und nach erfolgter Komplexbildung zwischen Antithrombin III und der jeweiligen Serinprotease wieder abgetrennt. Diese Affinität zwischen Antithrombin III und Heparin macht man auch im großtechnischen Maßstab bei der Herstellung von Antithrombin III-Konzentraten nutzbar.

Antithrombin III-Mangelzustände sind bei einer Vielzahl von Erkrankungen nachgewiesen[33]:

1. Angeboren
   Die Häufigkeit eines hereditär bedingten Antithrombin III-Mangels ist mit etwa 1:10.000 Einwohner bezogen auf die Bundesrepublik Deutschland einzuschätzen.
2. Renaler Eiweißverlust
   Aufgrund seines Molekulargewichtes von 64.500 Dalton wird Antithrombin III bei Vorliegen einer Glomerulopathie mit dem Urin ausgeschieden.
3. Proteinsynthesestörungen in der Leber
   Erkrankungen, die die Eiweißsynthese in der Leber einschränken, führen zwangsläufig zu Antithrombin-III-Mangelzuständen. Hierzu zählen sowohl akute Krankheitsbilder wie z. B. toxische Schädigungen der Leber als auch chronische Erkrankungen, wie Umbau.
4. Aktivierung des deren Folge ein v Gerinnungsfaktor steht. Hierzu ist intravasale Koagu nen.

Isolierungsmethode

Die Isolierung von III erfolgt aus Spen Zentrifugieren in kor sche Bestandteile aufg

Durch Nachweis von Anti-HTLV-III beim wird eine Verunrein mit Hepatitis-B und hend ausgeschlossen, daß HTLV-III-Carri körperbefund existier fahrens der Hitzeinakt die thermolabilen H III-Viren zerstört. Al tuelle Erkrankung des A/Non-B-Hepatitis GPT herangezogen.

Die Isolierung des An Plasmapool erfolgt m chromatographie an Sepharose. Es handel Form der Chromato nach ihrer Biospezifi Oberfläche des Sephar unlöslich fixiert, die trum spezifisch mit de tein reagieren können. tithrombin III bedient sauren Mukopolysacch nen isoelektrischen Pu als Ligand. Die stärks menschlichen Proteine se hat jedoch das Anti sorption der Probe an muß unter solchen Be

krankungen, wie etwa ein cirrhotischer Umbau.

4. Aktivierung des Gerinnungssystems, in deren Folge ein vermehrter Verbrauch von Gerinnungsfaktoren und Inhibitoren entsteht. Hierzu ist z. B. die disseminierte intravasale Koagulopathie (DIC) zu rechnen.

Isolierungsmethoden für AT III

Die Isolierung von humanen Antithrombin III erfolgt aus Spenderblut, welches durch Zentrifugieren in korpuskuläre und plasmatische Bestandteile aufgetrennt wird.

Durch Nachweis von HB_S-AG, HB_S-AK und Anti-HTLV-III beim einzelnen Blutspender wird eine Verunreinigung des Plasmapools mit Hepatitis-B und HTL-III-Viren weitgehend ausgeschlossen, wenngleich bekannt ist, daß HTLV-III-Carrier mit negativem Antikörperbefund existieren. Mit Hilfe des Verfahrens der Hitzeinaktivierung werden jedoch die thermolabilen Hepatitis-B- und HTL-III-Viren zerstört. Als Marker für eine eventuelle Erkrankung des Spenders an der Non-A/Non-B-Hepatitis wird die Aktivität der GPT herangezogen.

Die Isolierung des Antithrombin III aus dem Plasmapool erfolgt mit Hilfe der Affinitätschromatographie an mit Heparin beladener Sepharose. Es handelt sich hierbei um eine Form der Chromatographie, die Proteine nach ihrer Biospezifität auftrennt. An der Oberfläche des Sepharose-Gels sind Liganden unlöslich fixiert, die mit ihrem aktiven Zentrum spezifisch mit dem abzutrennenden Protein reagieren können. Zur Isolierung des Antithrombin III bedient man sich des sehr stark sauren Mukopolysaccharids Heparin, das einen isoelektrischen Punkt bei pH 1 aufweist, als Ligand. Die stärkste Affinität von allen menschlichen Proteinen zur Heparinsepharose hat jedoch das Antithrombin III. Die Adsorption der Probe an die Liganden des Gels muß unter solchen Bedingungen geschehen, bei denen eine Komplexbildung zwischen dem in der Probe enthaltenen Antithrombin III und dem Ligand möglich ist.

Bei der Elution wird das Milieu des Gels kontinuierlich so verändert, daß sich die Komplexe auflösen.

Man kann hierzu die Ionenstärke, den pH-Wert oder die Temperatur verändern. Für die Verdrängung des Antithrombin III aus der Heparinbindung wurden auch mit Erfolg Substanzen verwendet, die basische Eigenschaften haben. Bei der Isolierung von Antithrombin III sind bisher zumeist Natriumchloridgradienten zur Desorption verwendet worden[22,24].

Antithrombin III wird dabei wegen seiner hohen Affinität zur Heparinsepharose als letztes Protein desorbiert. *Miller-Andersson* et al.[22] beschrieben eine Antithrombin III-Desorption bei einer Natriumchloridkonzentration von etwa 1,0 mol/l für die Isolierung aus Plasma.

Wir fanden bei Versuchen mit hochreinen Antithrombin III-Präparaten eine Desorption bei einer Natriumchloridkonzentration zwischen 0,65 und 0,9 mol/l, wobei die optimale Salzkonzentration in geringem Umfang präparateabhängig war[29].

Es konnte gezeigt werden, daß für die beiden erforderlichen aufeinanderfolgenden Chromatographieprozesse nicht unbedingt zwei Säulen mit verschiedenen Gelen verwendet werden müssen, sondern nur eine, sofern die Heparinsepharose zwischen den Fraktionierungsprozessen mit 6-molarem Harnstoff regeneriert wird. Eine in dieser Weise vorbehandelte Heparinsepharose erbrachte höhere Ausbeuten an Antithrombin III als eine nicht vorbehandelte.

Da das Antithrombin III bei einer Natriumchloridkonzentration zwischen 0,8 und 0,9 mol/l eluiert wird, wurde bei der zweiten Chromatographie in Stufen eluiert. Die erste Desorption erfolgte bei einer Natriumchlo-

rid-Konzentration von 0,5 mol/l. Bei diesem Schritt wurden ungebundene oder leicht gebundene Proteine desorbiert. Antithrombin III selber bleibt unter diesen Bedingungen vollständig an die Matrix gebunden. Die Desorption des Antithrombin III erfolgte mittels eines schnell ansteigenden Gradienten zwischen 0,5 und 1,5 mol/l Natriumchlorid. Nach diesem Trennvorgang ist die Konzentration des Antithrombin III etwa doppelt so hoch wie die im Plasma.

Da die biologische Aktivität des Antithrombin III bei hoher Ionenstärke rasch abnimmt, ist es wichtig, das Protein in einen Puffer mit niedriger Ionenstärke zu überführen. Im Technikumsmaßstab hat sich hierfür die Dialyse in semipermeablen Schläuchen am besten bewährt.

Unkritisch scheint der pH-Wert des Dialysepuffers zu sein, er kann in einem Bereich zwischen 6,5 und 8,5 schwanken.

Die Herstellung von Antithrombin III-Konzentraten für therapeutische Zwecke beruht im Grunde auf den oben geschilderten Verfahren und kann großtechnisch realisiert werden: Nach Abtrennung der korpuskulären Bestandteile des Spenderblutes durch scharfes Zentrifugieren wird das Plasma bei −80°C schockgefroren. Hieran schließt sich ein 24 Stunden dauernder Auftauprozeß bei +4°C an, wonach durch scharfes Zentrifugieren das Kryopräzipitat gewonnen wird. Der an Fibrinogen und Faktor VIII verarmte Überstand wird an Ionenaustauscher absorbiert, um die Gerinnungsfaktoren II, VII, IX, X und das Protein C zu entfernen. Das im Überstand befindliche Antithrombin III wird nun an Heparinsepharose absorbiert. Das Antithrombin III-Konzentrat wird durch stufenweise Elution, Dialyse und abschließende Gefriertrocknung gewonnen. Neuere Untersuchungen haben gezeigt, daß der möglicherweise vom Gel miteluierte Heparinanteil der so gewonnenen Präparate extrem klein[36] und in der praktischen Anwendung bedeutungslos ist. Der nun noch vorhandene Überstand kann in die Albumin-bzw. Gammaglobulinproduktion überführt werden. Abbildung 1 zeigt diese großtechnischen Produktionsschritte in der Zusammenfassung.

Biochemische Charakterisierung des affinitätschromatographisch gewonnenen AT III

Der Nachweis der Reinheit des gewonnenen Antithrombin III wurde bisher[2,3,27,22] mit Hilfe der Gelelektrophorese, der SDS-Elektrophorese und der isoelektrischen Fokussierung geführt. Wir haben hierzu zwei weitere Methoden verwendet[28], das Chromatofokussing und die hochauflösende Flüssigkeitschromatographie. Mit Hilfe des Chromatofokussings kann ein Proteingemisch nach den isoelektrischen Punkten der einzelnen Eiweißbestandteile aufgetrennt werden. Dieser ist für Anti-

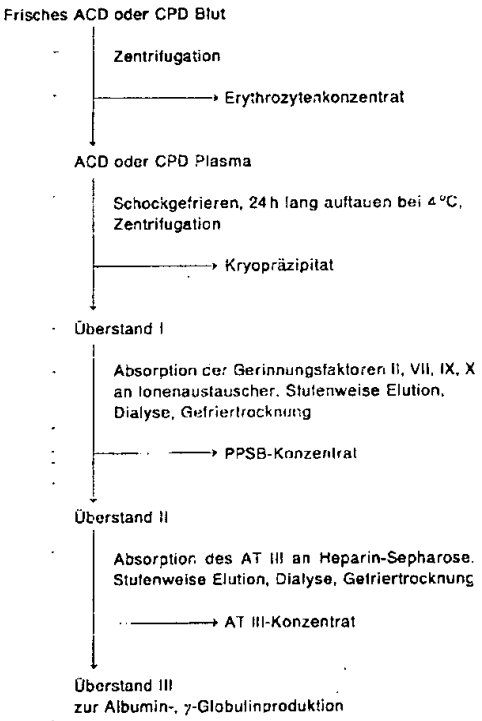

Abb. 1: Produktionsschritte bei der Herstellung von Antithrombin III-Konzentrat.

thrombin III bekannt u
ger[16] bei pH 5,11 sowi
zwei Antithrombin III
gegeben. Wir fanden
Punkt von pH 4,5 für
parinsepharose-Affinit
isolierte Antithrombin

Mit Hilfe des Chromat
einziger scharf begr
nachgewiesen werden.
ne Antithrombin III-I
einheitliches Antithro
wohl das Elutionsdiag
tätschromatographie
Dieses ist asymmetris
drei bis vier einzelnen
gen Fraktionen zusam
spielt dort jedoch das
Heparinsepharose eine

Mit Hilfe der hochauf
chromatographie (HPL
de zur Verfügung, die
ner Probenmengen ein
nes Proteingemisches
dieser Methode erweis
III-Präparation als hom

Das als hochreine Subs
thrombin III wurde
Aminosäuren zerlegt.
se des Hydrolysates er
72 Stunden, das Erge
Zeitpunkt von 0 Stunde
gebnisse wurden mit
Koide[19] und *Takahara*
belle 1).

Dieser Vergleich zeigt
Aminosäuren Serin un
Analysen nahezu gleich
sen ergaben für das A
hohen Anteil der saure
ragin- und Glutaminsäu
Aminosäuren ist Lysin
tration vertreten.

Wir fanden abweichend
chern in der PAA-G

Überstand kann in die Al-
Gammaglobulinproduktion
Abbildung 1 zeigt diese
Produktionsschritte in der

arakterisierung des affini-
hisch gewonnenen AT III

Reinheit des gewonnenen
wurde bisher[2,3,27,22] mit Hil-
horese, der SDS-Elektro-
elektrischen Fokussierung
n hierzu zwei weitere Me-
[28], das Chromatofokussing
sende Flüssigkeitschroma-
fe des Chromatofokussings
misch nach den isoelektri-
einzelnen Eiweißbestand-
erden. Dieser ist für Anti-

Blut

tion

⟶ Erythrozytenkonzentrat

Plasma

rieren, 24 h lang auftauen bei 4 °C,
tion

⟶ Kryopräzipitat

i der Gerinnungsfaktoren II, VII, IX, X
ustauscher. Stufenweise Elution,
efriertrocknung

⟶ PPSB-Konzentrat

i des AT III an Heparin-Sepharose.
ie Elution, Dialyse, Gefriertrocknung

⟶ AT III-Konzentrat

Globulinproduktion

chritte bei der Herstellung von
zentrat.

thrombin III bekannt und wird von *Heimburger*[16] bei pH 5,11 sowie von *Borsordi* et al.[6] für zwei Antithrombin III-Typen bei pH 4,9 angegeben. Wir fanden einen isoelektrischen Punkt von pH 4,5 für das von uns in der Heparinsepharose-Affinitätschromatographie isolierte Antithrombin III.

Mit Hilfe des Chromatofokussings konnte ein einziger scharf begrenzter Elutionsgipfel nachgewiesen werden. Durch die beschriebene Antithrombin III-Isolierung wird also ein einheitliches Antithrombin III gewonnen, obwohl das Elutionsdiagramm bei der Affinitätschromatographie eher dagegenspricht. Dieses ist asymmetrisch und scheint sich aus drei bis vier einzelnen Antithrombin III-haltigen Fraktionen zusammenzusetzen. Offenbar spielt dort jedoch das Bindungsverhalten an Heparinsepharose eine Rolle.

Mit Hilfe der hochauflösenden Flüssigkeitschromatographie (HPLC) stand eine Methode zur Verfügung, die bei Vorliegen nur kleiner Probenmengen eine exakte Trennung eines Proteingemisches ermöglicht. Auch mit dieser Methode erweist sich die Antithrombin III-Präparation als homogen.

Das als hochreine Substanz vorliegende Antithrombin III wurde hydrolytisch in seine Aminosäuren zerlegt. Die Aminosäureanalyse des Hydrolysates erfolgte nach 24, 48 und 72 Stunden, das Ergebnis wurde auf einen Zeitpunkt von 0 Stunden extrapoliert. Die Ergebnisse wurden mit Untersuchungen von *Koide*[19] und *Takahara* et al.[30] verglichen (Tabelle 1).

Dieser Vergleich zeigt, daß der Anteil der Aminosäuren Serin und Cystein bei allen drei Analysen nahezu gleich war. Alle drei Analysen ergaben für das Antithrombin III einen hohen Anteil der sauren Aminosäuren Asparagin- und Glutaminsäure. Von den basischen Aminosäuren ist Lysin in höchster Konzentration vertreten.

Wir fanden abweichend von anderen Untersuchern in der PAA-Gradienten-Gelelektrophorese ein Molekulargewicht von 70.000 Daltons. Verunreinigungen waren mit dieser Technik gleichfalls nicht erkennbar (Abb. 2).

Tabelle 1 Aminosäurengesamtanalyse von AT III. Vergleichende Gegenüberstellung fremder und eigener Untersuchungsergebnisse

Aminosäure	mol Aminosäure / mol Glycoprotein AT III		
	Koide (19)	*Takahara* (30)	eigene Analyse
ALA	29,1	32,5	42
ARG	20,3	23,5	35
ASP/ASN	44,2	47,7	59
CYS	6,1	6,1	5
GLU/GLN	49,6	54,1	68
GLY	20,0	19,1	22
HIS	6,2	5,3	7
ILE	20,5	21,1	25
LEU	38,0	39,5	54
LYS	32,4	36,7	54
MET	10,9	10,9	4
PHE	24,1	33,7	32
PRO	23,1	21,9	12
SER	32,8	32,9	32
THR	23,4	25,5	36
TRP	3,8	6,3	15
TYR	10,1	7,3	10
VAL	28,9	27,5	25

Das Gesamtmolekulargewicht bezieht sich auf das Glykoprotein Antithrombin III. Nach *Borsordi* et al.[6] ist der Saccharidanteil im Antithrombin III variabel und von der Vorbehandlung abhängig. Es werden für den Saccharidanteil Werte von 10 bis 17% angegeben[6,19,30].

Hierauf könnten die quantitativen Unterschiede in der Aminosäuren-Totalanalyse beruhen. Im Unterschied zu unseren Untersuchungen hat nämlich *Koide* ein aus mehreren aufeinanderabfolgenden Schritten bestehendes Isolierungsverfahren für Antithrombin III gewählt, wobei wesentlich mehr Veränderungen am Antithrombin III-Molekül zu erwarten sind, als bei der sehr schonenden Methode der Heparinsepharose-Affinitätschromatographie.

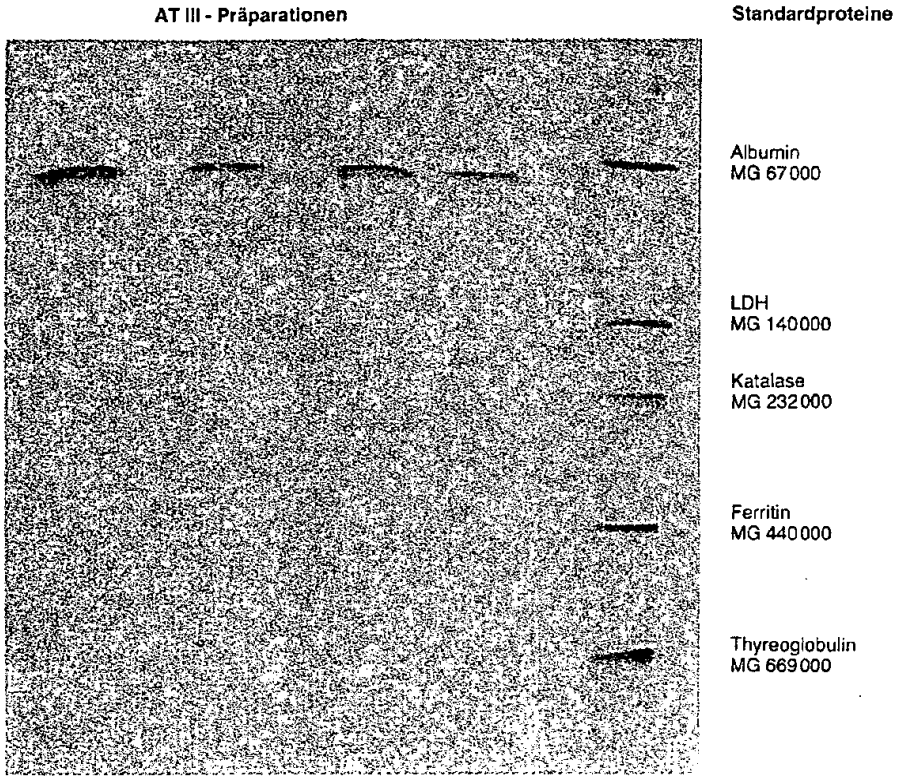

Abb. 2: PAA-Gradientengelelektrophorese von AT III.

Die Konzentrationsbestimmung des hochreinen Antithrombin III erfolgte mit Hilfe der radialen Immundiffusion und der spektralphotometrischen Konzentrationsbestimmung des Gesamteiweißes bei einer Wellenlänge von 280 nm. Dies war möglich, da das Antithrombin III als Reinsubstanz vorlag.

Beim Vergleich der Ergebnisse beider Methoden zeigte sich, daß die Konzentrationsmessungen mit Hilfe der radialen Immundiffusion doppelt so hohe Ergebnisse erbrachten, als mit der spektralphotometrischen Gesamteiweißbestimmung. Dieses Phänomen, das bereits von *Murano* et al.[24] beschrieben wurde, tritt dann auf, wenn hochgereinigtes Antithrombin III verwendet wird. Bei der radialen Immundiffusion werden Agarplatten mit spezifischen Antiseren gegen Antithrombin III eingesetzt. Die Ursache für das oben beschriebene Phänomen muß in einem unterschiedlichen Diffusionsverhalten von hochreinem Antithrombin III und dem im Plasma enthaltenen Antithrombin III gesehen werden. Sehr wahrscheinlich erfolgt bei Plasmaproben eine Behinderung der Diffusion des Antithrombin III im Agargel durch andere Proteine. Veränderungen am Antithrombin III-Molekül scheiden aus, da das hochreine Antithrombin III seine biologische Aktivität behält und diese ein außerordentlich empfindlicher Parameter für Veränderungen am Molekül ist.

Enzymkinetische
Wechselwirkung z
und aktivierten Se

Wie bereits eingan
biologische Funkti
der Inhibition vo
Mit Hilfe klassisch
zum Jahre 1982 d
Antithrombin III-
tet worden[27,31]:

1. Die Inhibitions
was in der Beze
thrombin zum A
2. Der Enzym-In
iert unter phys
nicht.
3. Die Geschwindi
rung wird durch

Exakte kinetische
zwischen Serinpro
und Heparin ware
jedoch nicht erhob
blieb die Frage offe
Serinproteasen im
oder einer non-kon
tiviert.

In der Literatur lag
kompetitiven Mech
und *Damus*[27] kon
daß die Inaktivie
durch eine Bindung
ter Einbeziehung ei
bitor an das Serin
Proteasen erfolgt.

Dies ist nicht nur a
Kompetition sonde
daß das Antithrom
einer spezifischen
wird, an der Argini
wendung chromoge
tersuchten wir dahe
von Faktor Xa dur
den Einfluß von
mung[34].

Standardproteine

Albumin
MG 67000

LDH
MG 140000

Katalase
MG 232000

Ferritin
MG 440000

Thyreoglobulin
MG 669000

endet wird. Bei der radialen
erden Agarplatten mit spe-
n gegen Antithrombin III
sache für das oben beschrie-
uß in einem unterschiedli-
erhalten von hochreinem
and dem im Plasma enthal-
n III gesehen werden. Sehr
olgt bei Plasmaproben eine
Diffusion des Antithrombin
ch andere Proteine. Verän-
ntithrombin III-Molekül
as hochreine Antithrombin
ie Aktivität behält und diese
h empfindlicher Parameter
i am Molekül ist.

Enzymkinetische Charakterisierung der Wechselwirkung zwischen AT III, Heparin und aktivierten Serylproteasen

Wie bereits eingangs festgestellt, besteht die biologische Funktion des Antithrombin III in der Inhibition von aktiven Serinproteasen. Mit Hilfe klassischer Testverfahren waren bis zum Jahre 1982 drei typische Merkmale der Antithrombin III-Enzyminteraktion erarbeitet worden[27,31]:

1. Die Inhibitionsgeschwindigkeit ist gering, was in der Bezeichnung progressives Antithrombin zum Ausdruck gebracht wird.
2. Der Enzym-Inhibitor-Komplex dissoziiert unter physiologischen Bedingungen nicht.
3. Die Geschwindigkeit der Enzyminaktivierung wird durch Heparin beschleunigt.

Exakte kinetische Daten der Wechselwirkung zwischen Serinproteasen, Antithrombin III und Heparin waren bis zu diesem Zeitpunkt jedoch nicht erhoben worden. Insbesondere blieb die Frage offen, ob Antithrombin III die Serinproteasen im Sinne einer kompetitiven oder einer non-kompetitiven Inhibition inaktiviert.

In der Literatur lagen Hinweise für einen nonkompetitiven Mechanismus vor[4]. *Rosenberg* und *Damus*[27] konnten jedoch 1973 zeigen, daß die Inaktivierung der Serinproteasen durch eine Bindung des Antithrombin III unter Einbeziehung eines Arginylrestes im Inhibitor an das Serin des aktiven Zentrums der Proteasen erfolgt.

Dies ist nicht nur als früher Hinweis auf eine Kompetition sondern auch dafür zu werten, daß das Antithrombin durch die Protease an einer spezifischen Peptidsequenz gespalten wird, an der Arginin beteiligt ist. Unter Verwendung chromogener Peptidsubstanzen untersuchten wir daher den Typ der Hemmung von Faktor Xa durch Antithrombin III und den Einfluß von Heparin auf diese Hemmung[34].

Unter Versuchsbedingungen, bei denen eine Kompetition äquimolarer Konzentrationen von Inhibitor und chromogenem Peptidsubstrat um das aktive Zentrum von Faktor Xa ermöglicht wurde, konnte ein kompetitiver Hemmtyp, erkennbar an der unveränderten Maximalgeschwindigkeit bei gestiegener Michaelis-Menten-Konstante (K_M), nachgewiesen werden (Abb. 3).

Hierbei ergibt sich eine lineare Abhängigkeit der K_M von der Antithrombin III-Konzentration.

Frühere Untersuchungen zeigten, daß Heparin die Hemmung der Serinproteasen durch Antithrombin III beschleunigt[5]. Da wir zudem nachweisen konnten, daß dadurch die Kapazität von Antithrombin III nicht verändert wurde, setzten wir Heparin als biologischen Katalysator der Reaktion ein, wobei die Erhöhung der K_M gegenüber der reinen Antithrombin III-Hemmung signifikant gesteigert wurde.

Abbildung 4 zeigt, daß die K_M in Abhängigkeit von der Heparinaktivität bei konstanter Antithrombin III-Aktivität eine gegen einen Grenzwert strebende Funktion ist. Hierbei genügen bereits geringe Heparinmengen, um bei hoher Antithrombin III-Ausgangskonzentration eine deutliche Effektivierung der Hemmung von Faktor Xa zu bewirken. Das Maximum der Heparinwirkung wird bei einem Verhältnis der Einheiten Antithrombin III zu Heparin von 1:5 erreicht.

Eine weitere Konzentrationssteigerung von Heparin bewirkt keine stärkere Erhöhung der K_M.

Als technisch mögliches Maximum fanden wir ein molares Verhältnis von Heparin zu Antithrombin von 1:20. Bei größeren Heparinkonzentrationen trat eine Fällungsreaktion ein, wahrscheinlich bedingt durch das verwendete chromogene Peptidsubstrat S 2222[15,12].

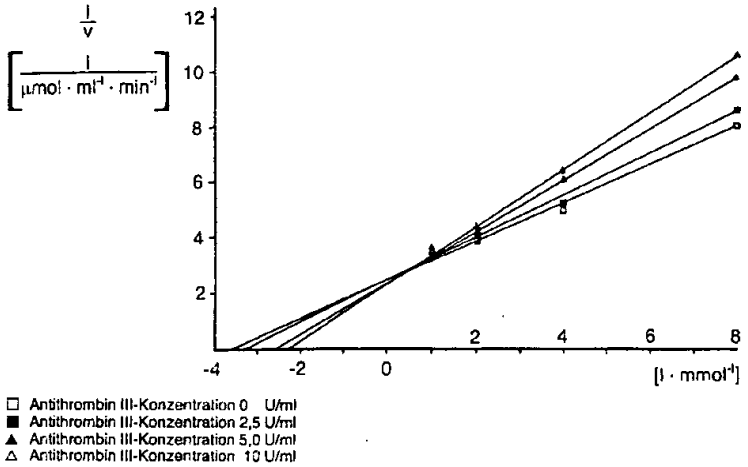

Abb. 3: Abhängigkeit der Reaktionsgeschwindigkeit von F X_a mit dem Kunstsubstrat S 2222 von der Substratkonzentration in doppelt reziproker Auftragung nach *Lineweaver-Burk* bei verschiedenen Antithrombin III-Konzentrationen.

Diese Tatsache und der e-funktionelle Verlauf der Kurve in der Abbildung 4 weisen darauf hin, daß bei dieser Heparinkonzentration die von *Rosenberg*[27] beschriebenen Lysinbindungen an AT III für Heparin abgesättigt sind.

In einem weiteren Versuch konnte der kompetitive Hemmtyp für wechselnde AT III-Konzentrationen bei maximaler Heparinaktivierung nachgewiesen werden. In diesem Fall besteht eine lineare Abhängigkeit der K_M von der AT III-Konzentration.

Beim Vergleich der K_M-Vergrößerung als Funktion der AT III-Konzentration mit und ohne Heparin wird die katalytische Funktion

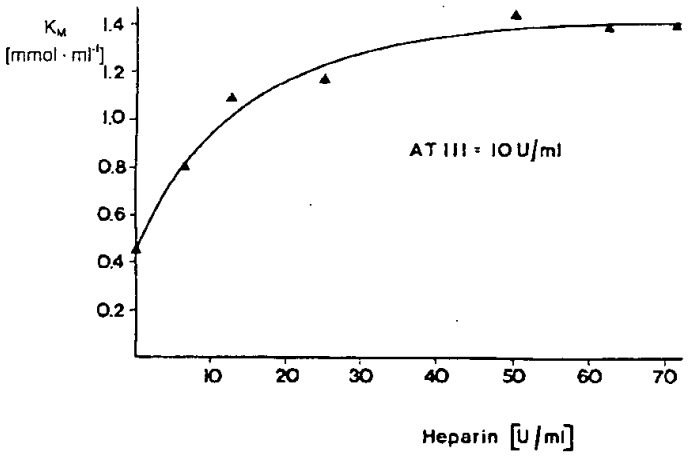

Abb. 4: K_M als Funktion der Heparinkonzentration bei konstantem Antithrombin III-Spiegel.

von Heparin sowie
der eingesetzten AT
lich (Abb. 5).

Die Wirkung von He
risiert durch:

1. eine Effektivierun
der Faktor Xa-H
und
2. eine Abhängigkeit
zentration.

Der kompetitive Hem
sche Rolle von Hepa
Auftragung der von
einem Plot nach *Dixo*
dabei gefundene K_I
zentration an, die erfo
zymatisch katalysiert
zu hemmen[9] oder – a
K_I ist die Dissoziation
Inhibitor-Komplexes
ner K_I ist, desto wenig
tigt, um einen definier
zu erreichen.

Bei der reinen AT III-
der Schnittpunkt S
oberen Quadranten,
Hemmung hinweist.
AT III/ml.

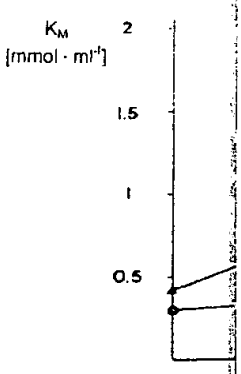

Abb. 5: Abhängigkeit der K

von Heparin sowie deren Abhängigkeit von der eingesetzten AT III-Konzentration deutlich (Abb. 5).

Die Wirkung von Heparin ist damit charakterisiert durch:

1. eine Effektivierung und Beschleunigung der Faktor Xa-Hemmung durch AT III und
2. eine Abhängigkeit von der AT III-Konzentration.

Der kompetitive Hemmtyp und die katalytische Rolle von Heparin konnten durch eine Auftragung der von uns erhobenen Daten in einem Plot nach *Dixon* bestätigt werden. Die dabei gefundene K_I gibt die Inhibitor-Konzentration an, die erforderlich ist, um eine enzymatisch katalysierte Reaktion halbmaximal zu hemmen[9] oder – anders ausgedrückt – die K_I ist die Dissoziationskonstante des Enzym-Inhibitor-Komplexes[26]. Das bedeutet, je kleiner K_I ist, desto weniger Inhibitor wird benötigt, um einen definierten Grad der Hemmung zu erreichen.

Bei der reinen AT III-Hemmung (Abb. 6) lag der Schnittpunkt S der Geraden im linken oberen Quadranten, was auf eine kompetitive Hemmung hinweist. Die K_I betrug 19,64 U AT III/ml.

Bei maximaler Beschleunigung durch Heparin (Abb. 7) blieb die Lage des Schnittpunktes S erhalten, lediglich die K_I sank auf einen Wert von 5,2 U AT III/ml. Die in beiden Fällen gefundenen Maximalgeschwindigkeiten sind sowohl untereinander als auch mit den nach dem Verfahren von *Lineweaver* und *Burk* ermittelten Werten identisch. Daraus geht hervor:

1. Die Hemmung von Faktor Xa durch AT III ist kompetitiv.
2. Dies ändert sich nicht unter Heparinkatalyse.
3. Heparin senkt die K_I und wirkt daher als Katalysator der Hemmung von Faktor Xa durch AT III.

Die Heparin-AT III-Wechselwirkung ist in Abbildung 8 zusammenfassend dargestellt.

In Zeile 1 ist zunächst die enzymatisch katalysierte Substratspaltung in P_1 und P_2 gezeigt. Nach erfolgter Katalyse steht das Enzym erneut zur Verfügung. Nach Zugabe des Inhibitors AT III (Zeile 2) wird jedoch kontinuierlich im Sinne einer dead-end-Inhibition Enzym aus dem System eliminiert, wobei sich aber ein zunächst noch rückdissoziabler Komplex (EI) mit der Dissoziationskonstanten K_I ausbildet.

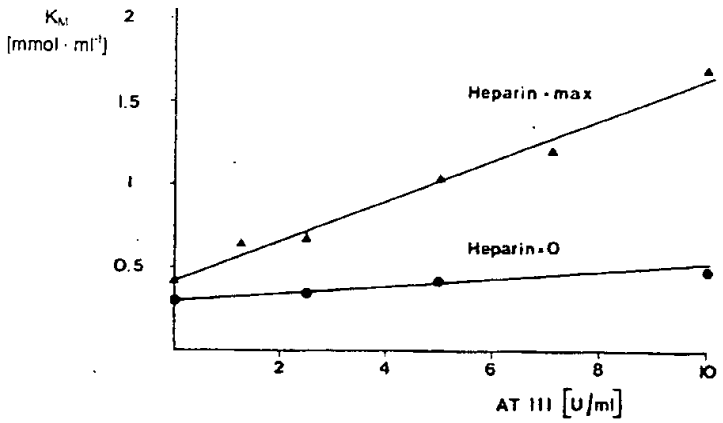

Abb. 5: Abhängigkeit der K_M von der AT III-Konzentration mit und ohne Heparinkatalyse.

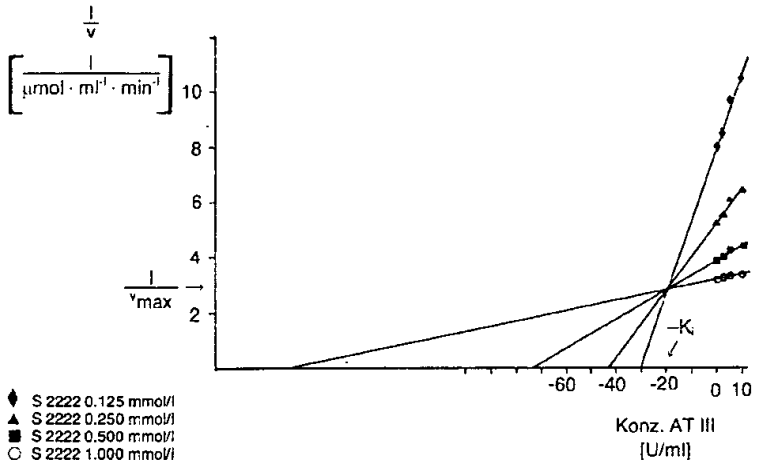

Abb. 6: Darstellung der Hemmung von Faktor X, durch Antithrombin III nach *Dixon* bei vier verschiedenen Substratkonzentrationen.

Diese Hemmung ist initial kompetitiv. Innerhalb kürzester Zeit wechselt sie scheinbar zum non-kompetitiven Typ. Diese Beobachtung ist jedoch virtuell, da durch das ständige Verarmen des Systems an Faktor Xa eine Erniedrigung der Maximalgeschwindigkeit eintritt, die eine non-kompetitive Hemmung vortäuscht. Dies ist jedoch nicht mehr Ausdruck einer unmittelbaren Konkurrenz zwischen AT III und dem chromogenen Peptidsubstrat um den Faktor Xa, sondern der irreversiblen Komplexbildung zwischen Inhibitor und Enzym. Nach Zugabe von Heparin (Zeile 3) bilden sich sehr schnell Inhibitor-Heparin-Komplexe mit der Assoziationskonstante K_A[32].

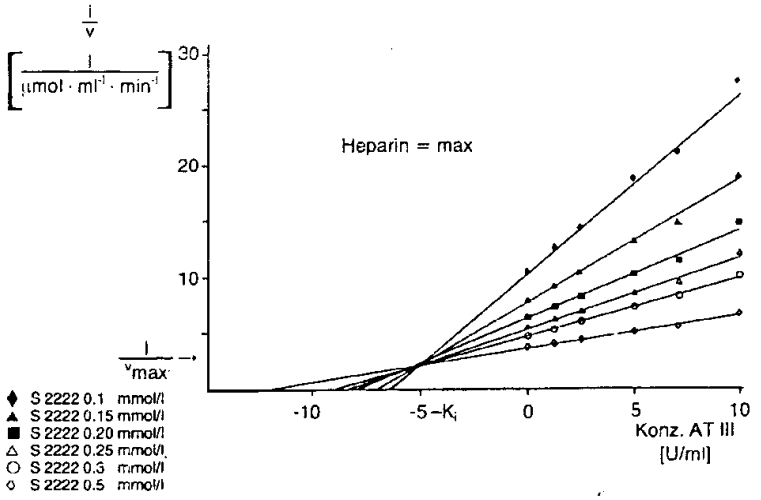

Abb. 7: Darstellung der Hemmung von Faktor X_a durch AT III unter Heparinkatalyse nach *Dixon* bei sechs verschiedenen Substratkonzentrationen.

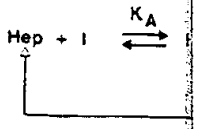

Abb. 8: Wirkungsprin

Diese haben eine
reine Inhibitor, w
fektiviert wird un
tition betont wird
tigen, nicht meh
Inhibitor-Komple
erneuten Katalyse

**Möglichkeiten d
Konzentrat**

Die physiologisch
bin III bestimmt
Einsatz von AT II

Neben der Thera
stände hat sich di
AT III-Konzentr
schädigungen,
Thrombembolie-I
zur Therapie der
Koagulopathie be

Die Verbrauchsk
möostase-Störung
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nung einhergeht (
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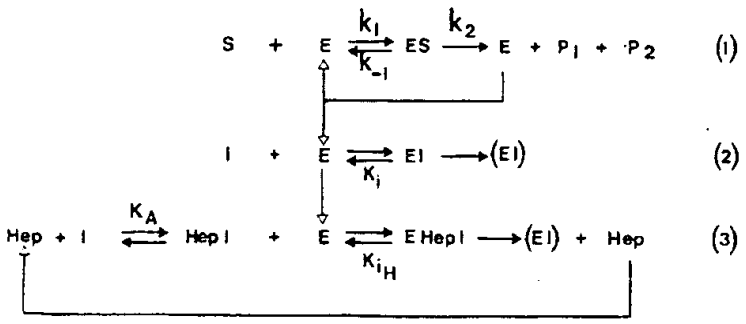

Abb. 8: Wirkungsprinzip von AT III und Heparin.

Diese haben eine wesentlich kleinere K_I als der reine Inhibitor, wobei die Hemmung stark effektiviert wird und der Charakter der Kompetition betont wird. Nach Bildung des endgültigen, nicht mehr rückdissoziablen Enzym-Inhibitor-Komplexes (EI) steht Heparin zur erneuten Katalyse zur Verfügung.

Möglichkeiten der Therapie mit AT III-Konzentrat

Die physiologische Wirkung von Antithrombin III bestimmt auch den therapeutischen Einsatz von AT III-Konzentraten.

Neben der Therapie hereditärer Mangelzustände hat sich die Substitutionstherapie mit AT III-Konzentraten bei toxischen Leberschädigungen, zur Effektivierung der Thrombembolie-Prophylaxe und besonders zur Therapie der disseminierten intravasalen Koagulopathie bewährt[8].

Die Verbrauchskoagulopathie ist eine Homöostase-Störung des Gerinnungssystems, die mit einer initialen Aktivierung der Gerinnung einhergeht (Abb. 9). Bereits in dieser ersten Phase kann es durch Komplexbildung der aktivierten Gerinnungsfaktoren mit AT III zu einem Inhibitor-Mangel kommen. Eine isolierte Heparintherapie könnte diesen Aktivierungszustand des Gerinnungssystems nicht beenden, da Heparin alleine keine antikoagulatorische Wirkung besitzt, sondern lediglich als Katalysator des AT III wirkt.

Gelingt es durch therapeutische Bemühungen nicht, bereits in der Phase I der Verbrauchsreaktion das Gerinnungssystem zu kompensieren oder nimmt das Krankheitsbild einen perakuten Verlauf, entwickelt sich das Stadium II der DIC (Abb. 10). Dieses ist klinisch durch das Auftreten von Schockorganen gekennzeichnet, da die nun massenhaft entstandenen Fibrin- und Thrombozytenaggregate die Mikrostrombahn in Lunge, Leber und Niere verstopfen.

Das Gleichgewicht zwischen dem Inhibitor AT III und den Gerinnungsfaktoren verschiebt sich weiter zuungunsten des Inhibitors, so daß in diesem Krankheitsstadium die Substitutionstherapie dringlich indiziert ist.

Dies gilt auch für das Finalstadium einer DIC (Stadium III), das durch eine reaktive Hyperfibrinolyse bei total dekompensierter Hämostase gekennzeichnet ist[35] (Abb. 11).

Antithrombin III ist also der wichtigste und derzeitig am besten charakterisierte Inhibitor des menschlichen Blutgerinnungssystems und hauptverantwortlich für die Aufrechterhaltung einer physiologischen Homöostase. Die Entwicklung von Isolierungsmethoden zur Reindarstellung dieses Proteins hat Hochkon-

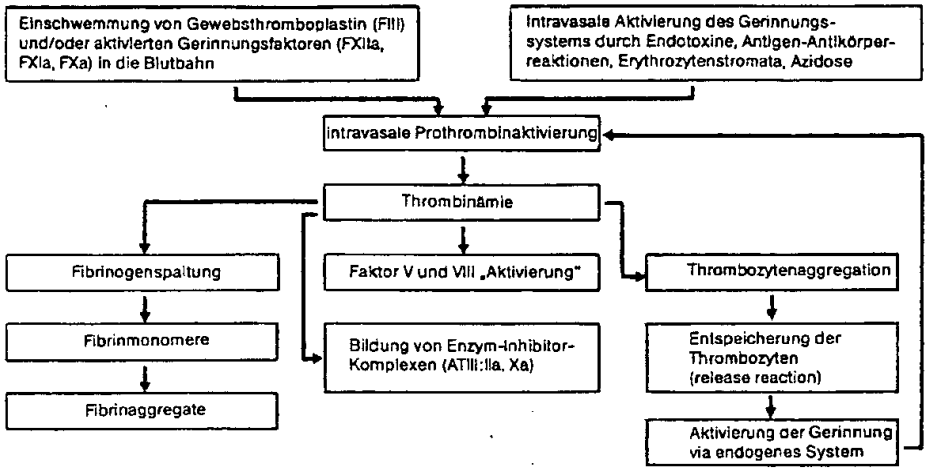

Abb. 9: Pathomechanismus des ersten Stadiums der Verbrauchsreaktion (Hyperkoagulämie).

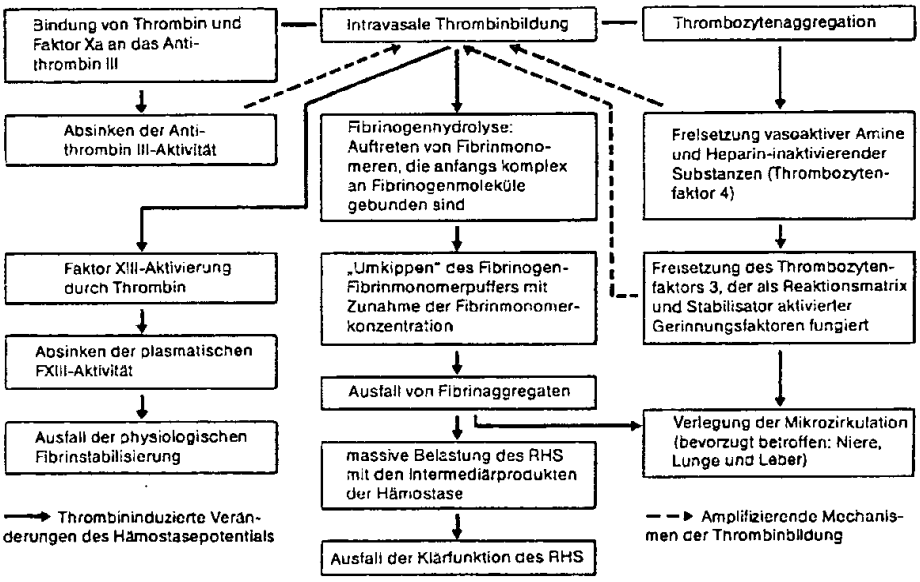

Abb. 10: Pathomechanismus des zweiten Stadiums der Verbrauchsreaktion (Faktorenverbrauch).

Lokale Hyperfibrinoly
Aktivatoren des Plasm
mus, die verlegte Mikro
zu befreien)
↓
Verschleppung von Pl
in die Zirkulation
↓
Fibrinogenhydrolyse d
↓
Auftreten hochmolekul
↓
Induktion des Fibrinke
spaltprodukt Y
↓
Totale Ungerinnbarkeit
↓
Hypovolämischer Schi
neigung

Abb. 11: Pathomechan
Verbrauchsreaktion
reaktiver Hyperfibrinoly

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systems gezielter u
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Literatur

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4 *Aoki, N.* & *Igarash*
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5 *Björk, I.* & *Norden*
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non Stoechiometric
Biochem. *68*, 507–51

6 *Borsordi, A.* & *Na*
roheterogenity of h
Haematol. *39* (1), 121

des Gerinnungs-
xine, Antigen-Antikörper-
instromata, Azidose

rombozytenaggregation

itspeicherung der
rombozyten
elease reaction)

ktivierung der Gerinnung
a endogenes System

gulämie).

mbozytenaggregation

setzung vasoaktiver Amine
Heparin-inaktivierender
stanzen (Thrombozyten-
or 4)

zung des Thrombozyten-
3, der als Reaktionsmatrix
abilisator aktivierter
ungsfaktoren fungiert

egung der Mikrozirkulation
orzugt betroffen: Niere,
ge und Leber)

Amplifizierende Mechanis-
r Thrombinbildung

nverbrauch).

Lokale Hyperfibrinolyse induziert durch zellständige Aktivatoren des Plasminogens (Versuch des Organismus, die verlegte Mikrozirkulation von Mikrothrombosen zu befreien)

↓

Verschleppung von Plasmin und Fibrinspaltprodukten in die Zirkulation

↓

Fibrinogenhydrolyse durch Plasmin intravasal

↓

Auftreten hochmolekularer Fibrinspaltprodukte

↓

Induktion des Fibrinkettenabbruchs durch das Fibrinspaltprodukt Y

↓

Totale Ungerinnbarkeit des Blutes

↓

Hypovolämischer Schock bei schwerster Blutungsneigung

Abb. 11: Pathomechanismus des dritten Stadiums der Verbrauchsreaktion (Verbrauchskoagulopathie bei reaktiver Hyperfibrinolyse).

zentrate für den therapeutischen Einsatz erbracht, mit deren Hilfe es möglich geworden ist, schwerste Entgleisungen des Gerinnungssystems gezielter und effektiver als dies früher möglich war zu therapieren.

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V. Therapeuti

The Treatment of C

G. E. VOGEL

II. Medizinische Klinik und P
Ismaninger Straße 22, D-8000

Summary

There has been a transfo
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Zusammenfassung

Das Syndrom der Verbr
auf dem gesamten Gebie
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Möglichkeit, die Balance

Key Words: Consumpt
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The Transformation in
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coagulopathy have unde
tion due to the biochem
physiology of coagulatio
methods made possible b
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(HG2) with BXa(-GD) slowed the inhibition of VIIa/TF after the addition of native Xa. The results are consistent with the hypothesis that inhibiton of VII(a)/TF involves the formation of a VIIa-TF-Xa-LACI complex that requires the GD of Xa. Because the GD contains the alpha-carboxyglutamic acids required for the Casup 2sup +-dependent binding of factor Xa to phospholipid surfaces, the results also suggest that Casup 2sup + may be required for the native Xa-LACI complex to bind to and inhibit VII(a)/TF. LACI is a novel inhibitor that can rapidly affect feedback inhibition of the VIIa-Casup 2sup +-TF enzymatic complex after the generation of small amounts of Xa and probably plays an important role in the regulation of the in vivo coagulation.

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96772 87175579 PMID: 3031657
Isolation of the tissue factor inhibitor produced by HepG2 hepatoma cells.
Broze G J; Miletich J P
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Apr 1987, 84 (7) p1886-90, ISSN 0027-8424 Journal Code: 7505876
Contract/Grant No.: HL34462; HL; NHLBI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Progressive inhibition of tissue factor activity occurs upon its addition to human plasma (serum). This process requires the presence of factor VII(a), facto-X(a), Ca2+, and another component in plasma that we have called the tissue factor inhibitor (TFI). A TFI secreted by HepG2 cells (human hepatoma cell line) was isolated from serum-free conditioned medium in a four-step procedure including CdCl2 precipitation, diisopropylphosphoryl-factor Xa affinity chromatography, Sephadex G-75 superfine gel filtration, and Mono Q ion-exchange chromatography. The purified TFI contained a predominant band at Mr 38,000 on NaDodSO4/polyacrylamide gel electrophoresis that comigrates with inhibitory activity. Like the activity present in plasma, this TFI requires the presence of factor VII(a), factor X(a), and Ca2+ to express inhibitory activity. Its specific activity (assuming an extinction coefficient of 10 at 280 nM, for a 1-cm path length through a 1% solution) was 9800 units/mg of protein, where 1 unit of TFI activity was defined as that present in 1 ml of normal pooled serum.
Record Date Created: 19870504
Record Date Completed: 19870504

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7/7/4 (Item 1 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

112135018 CA: 112(15)135018p JOURNAL
Isolation of the lipoprotein associated coagulation inhibitor produced by HepG2 (human hepatoma) cells using bovine factor Xa affinity chromatography
AUTHOR(S): Broze, George J., Jr.; Warren, Louise A.; Girard, James J.; Miletich, Joseph P.
LOCATION: Sch. Med., Washington Univ., St. Louis, MO, USA
JOURNAL: Thromb. Res. DATE: 1987 VOLUME: 48 NUMBER: 2 PAGES: 253-9
CODEN: THBRAA ISSN: 0049-3848 LANGUAGE: English
SECTION:
CA207003 Enzymes
CA213XXX Mammalian Biochemistry
IDENTIFIERS: lipoprotein assocd coagulation inhibitor affinity chromatog, blood lipoprotein assocd coagulation inhibitor purifn, protein inhibitor lipoprotein assocd coagulation purifn
DESCRIPTORS:
Blood-coagulation factors,LACI (lipoprotein-assocd. coagulation inhibitor)
...
of HepG2 cells, of human, affinity chromatog. in isolation of

VII, is frequently complicated by the development of neutralizing antibodies. The therapeutic potential of attenuated forms of the lipid-associated glycoprotein tissue factor, a known inhibitor of coagulation, was investigated as a factor VIII-bypassing activity. The protein moiety of tissue factor (Apo-TF) was partially purified and exhibited minimal procoagulant activity before relipidation in vitro. In pilot studies, Apo-TF injection into rabbits previously anticoagulated with an antibody to factor VIII was found to have a procoagulant effect. The efficacy of the material was further demonstrated when injection of Apo-TF in hemophilic dogs resulted in a normalization of the cuticle bleeding time. Little or no change in the blood parameters associated with disseminated intravascular coagulation was observed at lower doses, although mild to moderate effects were seen at higher doses. These data suggest a novel role for Apo-TF preparations as a potential therapeutic agent for hemophilics with antibodies to factor VIII once the potential thrombogenicity of such materials is evaluated.

RB1.C45

Phillip Gambell
9/24
Art Unit 1644

14/7/3 (Item 2 from file: 73)
DIALOG(R)File 73:EMBASE
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02350096 EMBASE No: 1983229100
Molecular markers of hemostatic disorders: Implications in the diagnosis and therapeutic management of thrombotic and bleeding disorders
Fareed J.; Bick R.L.; Squillaci G.; et al.
Loyola Univ. Med. Cent., Maywood, IL 60153 United States
Clinical Chemistry (CLIN. CHEM.) (United States) 1983, 29/9 (1641-1658)
CODEN: CLCHA
DOCUMENT TYPE: Journal
LANGUAGE: ENGLISH

With current technological advances, it is now possible to measure in <50 muL of plasma picomolar amounts of circulating products of platelet activation, products of protease activation related to coagulation and fibrinolytic pathways, and prostaglandin metabolites formed during a physiologic or pathologic process. Most of these markers, which circulate in blood in nanogram or picogram amounts per milliliter during or after pathologic activation, provide pertinent information on the status of a patient in terms of specificity and early detection, and will be of crucial value in the diagnosis of hemostatic defects and the management of newer antithrombotic drugs that cannot be monitored by currently available assays. Currently, sup 1sup 2sup 5I- and sup 3H-based simple radioimmunoassays are available for platelet factor 4, beta-thromboglobulin, fibrinopeptide A, Bbeta 15-42 related peptides, thromboxane Binf 2, and the prostaglandins 6-keto-PGF(inf 1alpha) and PGEinf 2. Nonisotopic methods such as enzyme-linked immunosorbent assays and fluoroimmunoassays are being developed. Serotonin and ADP, products of platelet activation, are measurable by liquid-chromatographic, immunoenzymatic, and spectrophotofluorometric methods. Analytical methods for fibrin split products (fragments D and E) and serine protease inhibitor complexes such as thrombin-antithrombin-III, factor Xa-antithrombin-III, and kallikrein-Cinf 1-esterase are also being developed. We have evaluated all of these methods and found them to be very sensitive to those components of endogenous activation of the hemostatic system listed above.

14/7/4 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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04998841 85305963 PMID: 3929705
Coronary bypass surgery in patients with circulating lupus anticoagulant.

Q11, N26

Philip Gambel
9/24/03
AU-1644

(HG2) with BXa(-GD) slowed the inhibition of VIIa/TF after the addition of native Xa. The results are consistent with the hypothesis that inhibiton of VII(a)/TF involves the formation of a VIIa-TF-Xa-LACI complex that requires the GD of Xa. Because the GD contains the alpha-carboxyglutamic acids required for the Casup 2sup +-dependent binding of factor Xa to phospholipid surfaces, the results also suggest that Casup 2sup + may be required for the native Xa-LACI complex to bind to and inhibit VII(a)/TF. LACI is a novel inhibitor that can rapidly affect feedback inhibition of the VIIa-Casup 2sup +-TF enzymatic complex after the generation of small amounts of Xa and probably plays an important role in the regulation of the in vivo coagulation.

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Broze G J; Miletich J P
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Apr 1987, 84 (7) p1886-90, ISSN 0027-8424 Journal Code: 7505876
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Record type: Completed
Progressive inhibition of tissue factor activity occurs upon its addition to human plasma (serum). This process requires the presence of factor VII(a), facto-X(a), Ca2+, and another component in plasma that we have called the tissue factor inhibitor (TFI). A TFI secreted by HepG2 cells (human hepatoma cell line) was isolated from serum-free conditioned medium in a four-step procedure including CdCl2 precipitation, diisopropylphosphoryl-factor Xa affinity chromatography, Sephadex G-75 superfine gel filtration, and Mono Q ion-exchange chromatography. The purified TFI contained a predominant band at Mr 38,000 on NaDodSO4/polyacrylamide gel electrophoresis that comigrates with inhibitory activity. Like the activity present in plasma, this TFI requires the presence of factor VII(a), factor X(a), and Ca2+ to express inhibitory activity. Its specific activity (assuming an extinction coefficient of 10 at 280 nM, for a 1-cm path length through a 1% solution) was 9800 units/mg of protein, where 1 unit of TFI activity was defined as that present in 1 ml of normal pooled serum.
Record Date Created: 19870504
Record Date Completed: 19870504

LACI----------laci----------

7/7/4 (Item 1 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

112135018 CA: 112(15)135018p JOURNAL
Isolation of the lipoprotein associated coagulation inhibitor produced by HepG2 (human hepatoma) cells using bovine factor Xa affinity chromatography
AUTHOR(S): Broze, George J., Jr.; Warren, Louise A.; Girard, James J.; Miletich, Joseph P.
LOCATION: Sch. Med., Washington Univ., St. Louis, MO, USA
JOURNAL: Thromb. Res. DATE: 1987 VOLUME: 48 NUMBER: 2 PAGES: 253-9
CODEN: THBRAA ISSN: 0049-3848 LANGUAGE: English
SECTION:
CA207003 Enzymes
CA213XXX Mammalian Biochemistry
IDENTIFIERS: lipoprotein assocd coagulation inhibitor affinity chromatog, blood lipoprotein assocd coagulation inhibitor purifn, protein inhibitor lipoprotein assocd coagulation purifn
DESCRIPTORS:
Blood-coagulation factors,LACI (lipoprotein-assocd. coagulation inhibitor)
...
of HepG2 cells, of human, affinity chromatog. in isolation of

Kelly J P; Thomas L; Moulder P V; Webb W R
Annals of thoracic surgery (UNITED STATES) Sep 1985, 40 (3) p261-3, ISSN 0003-4975 Journal Code: 15030100R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Clinical and laboratory experience with circulating lupus anticoagulant in 3 patients undergoing coronary artery bypass procedures is reported. This circulatory anticoagulant inhibits activation of prothrombin by the prothrombin activator complex (factor Xa, factor V, and phospholipid). The presence of lupus anticoagulant was initially detected because of a prolonged activated partial thromboplastin time and a normal or mildly prolonged prothrombin time. The 3 patients underwent uncomplicated coronary artery bypass grafting and experienced no abnormal bleeding postoperatively. The lupus anticoagulant is a rare cause of bleeding after open-heart surgery. It appears to be a problem only when an additional coagulation defect is present.
Record Date Created: 19851001
Record Date Completed: 19851001

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14/7/5 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

04938601 85245383 PMID: 4012669
Comparative effects of heparin and PK 10169, a low molecular weight fraction, in a canine model of arterial thrombosis.
Mestre M; Clairefond P; Mardiguian J; Trillou M; Le Fur G; Uzan A
Thrombosis research (UNITED STATES) May 15 1985, 38 (4) p389-99, ISSN 0049-3848 Journal Code: 0326377
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The comparative properties of heparin and PK 10169, a low molecular weight fraction, were studied using an antithrombotic test in anaesthetized dogs. The antithrombotic properties of the two compounds were evaluated by measuring inhibition of thrombus formation following transluminar stimulation of coronary artery with anodal current and by measuring anticoagulant properties, anti Xa and anti IIa activities. The results show that PK 10169 displayed significant antithrombotic activities above 0.625 mg/kg and was equipotent at 2.5 mg/kg s.c. with heparin 10 mg/kg s.c. No correlation could be observed between antithrombotic/anti Xa ratio of both compounds. Moreover it was shown that, unlike heparin, PK 10169 s.c. was devoid of obvious anticoagulant properties and induced a negligible anti IIa activity contrasting with a high anti Xa level. A similar dissociation between anti Xa and anti IIa activities was observed following i.v. administration of 2.5 mg/kg of PK 10169 but not with heparin. This low molecular weight heparin fraction might thus be regarded as a potential arterial antithrombotic agent devoid of appreciable anticoagulant effect.
Record Date Created: 19850812
Record Date Completed: 19850812

----------laci----------

----------laci----------

14/7/7 (Item 1 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

104202975 CA: 104(23)202975y JOURNAL
Isolation and characterization of human antithrombin III
AUTHOR(S): Trobisch, H.; Wuest, T.; Rustige, H.

Val. No.
4653911
Phillip Gamb
Art Unit 1644
9/24
TF
(COMPLETED)
1177405

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RB1.J5

STIC-ILL

From: Gambel, Phillip
Sent: Wednesday, September 24, 2003 4:48 PM
To: STIC-ILL
Subject: laci two

stic

please provide the following references to

phillip gambel
art unit 1644
308-3997

1644 mailbox 9e12

14/7/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

02121116 BIOSIS NO.: 000063036112
PENICILLIN INDUCED COAGULATION DISORDER
AUTHOR: ANDRASSY K; SCHERZ M; RITZ E; WALTER E; HASPER B; STORCH H; VOEMEL W
JOURNAL: LANCET 2 (7994). 1976 1039-1041. 1976
FULL JOURNAL NAME: Lancet
CODEN: LANCA
RECORD TYPE: Abstract

ABSTRACT: A coagulation disorder was seen after penicillin G administration (10 million units/day) in uremic patients and after high-dose penicillin G (40 million units/day) in patients with a normal glomerular filtration rate (5 patients after cardiac surgery). This disorder was characterized by prolongation of bleeding time, appearing immediately after penicillin G administration and persisting until 4 days after withdrawal of therapy; disturbance of collagen-induced and ristocetin-induced platelet aggregation; increase of antithrombin-III activity; and inhibition of factor-Xa activity. The inhibition of factor-Xa activity corresponded to that seen after low-dose heparin prophylaxis. The clinically latent coagulation disorder, when superimposed upon pre-existing coagulation abnormalities (uremia, treatment with anticoagulants) may cause severe bleeding, as observed in 1 patient with acute renal failure on hemodialysis.

14/7/2 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

03813081 EMBASE No: 1988262521
Factor VIII-bypassing activity of bovine tissue factor using the canine hemophilic model
O'Brien D.P.; Giles A.R.; Tate K.M.; Vehar G.A.
Department of Cardiovascular Research, Genetech, Inc., South San Francisco, CA 94080 United States
Journal of Clinical Investigation (J. CLIN. INVEST.) (United States) 1988, 82/1 (206-211)
CODEN: JCINA ISSN: 0021-9738
DOCUMENT TYPE: Journal
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

The bleeding disorder of hemophilia A currently treated by replacement therapy of the missing coagulation factor, factor

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     $0.01  TELNET
     $0.01  Estimated cost this search
     $0.29  Estimated total session cost   0.151 DialUnits

SYSTEM:OS  - DIALOG OneSearch
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         (c) 2003 BIOSIS
  File  73:EMBASE  1974-2003/Sep W2
         (c) 2003 Elsevier Science B.V.
  File 155:MEDLINE(R)  1966-2003/Sep W2
         (c) format only 2003 The Dialog Corp.
*File 155: Medline has been reloaded and accession numbers have
changed.  Please see HELP NEWS 155.
  File 399:CA SEARCH(R)  1967-2003/UD=13913
         (c) 2003 American Chemical Society
*File 399: Use is subject to the terms of your user/customer agreement.
Alert feature enhanced for multiple files, etc. See HELP ALERT.

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E1        3  AU=O(TILDE)UNPUU S.
E2        1  AU=O(TILDE)ZYILMAZ M.
E3        0 *AU=O(W)BRIEN DONOUGH ?
E4        1  AU=O+QUOT;NEILL, ANTHONY
E5        1  AU=O;CONNOR, M.
E6        1  AU=O;NEILL, HUGH ST. C.
E7        1  AU=O;RAIFEARTAIGH, C.
E8       10  AU=O-AE, SHIGERU
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E10       1  AU=O-BOATENG A.
E11       1  AU=O-BOATENG, A.
E12       1  AU=O-BRIEN C

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E9        1  AU=O BRIEN F H
E10       1  AU=O BRIEN F J
E11       1  AU=O BRIEN F V
E12       2  AU=O BRIEN G

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E3         0 *AU=O BRIEN D ?
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E6         4  AU=O BRIEN D J
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E9         4  AU=O BRIEN E M
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E11        3  AU=O BRIEN E T
E12        4  AU=O BRIEN F

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 1/3/1     (Item 1 from file: 5)
DIALOG(R)File   5:Biosis Previews(R)
(c) 2003 BIOSIS. All rts. reserv.

00539274   BIOSIS NO.: 000051129264
SELECTIVE REDUCTION OF INCARCERATED INGUINAL HERNIA
AUTHOR: KAUFFMAN H M JR; O BRIEN D P
JOURNAL: AMER J SURG 119 (6). 1970 660-673. 1970
CODEN: AJSUA
RECORD TYPE: Citation
? s (laci or lipoprotein(W)associated(w)coagulation)
           3391  LACI
         246954  LIPOPROTEIN
        2741504  ASSOCIATED
         212521  COAGULATION
            817  LIPOPROTEIN(W)ASSOCIATED(W)COAGULATION
      S2    4094  (LACI OR LIPOPROTEIN(W)ASSOCIATED(W)COAGULATION)
? s s2 and py<1989
Processing
           4094  S2
       25118864  PY<1989
      S3    517  S2 AND PY<1989
? s s2 and coagulation
           4094  S2
         212521  COAGULATION
      S4    1030  S2 AND COAGULATION
? s s4 and lipoprotein
           1030  S4
         246954  LIPOPROTEIN
      S5    1025  S4 AND LIPOPROTEIN
? s s2 and coagulation(20n)(treat? or inhibit? or suppress? or therap? or block? or
antagoni?)
Processing
Processing
Processing
           4094  S2
         212521  COAGULATION
        6164172  TREAT?
        3807036  INHIBIT?
         767919  SUPPRESS?
        5711282  THERAP?
        1204598  BLOCK?
        1006773  ANTAGONI?
          58229  COAGULATION(20N)(((((TREAT? OR INHIBIT?) OR SUPPRESS?) OR
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                THERAP?) OR BLOCK?) OR ANTAGONI?)
      S6    1028  S2 AND COAGULATION(20N)(TREAT? OR INHIBIT? OR SUPPRESS?
                OR THERAP? OR BLOCK? OR ANTAGONI?)
? s s6 and py=1987
            1028  S6
         1561212  PY=1987
      S7       4  S6 AND PY=1987
? t s7/7/all

 7/7/1     (Item 1 from file: 5)
DIALOG(R)File   5:Biosis Previews(R)
(c) 2003 BIOSIS. All rts. reserv.

05789432   BIOSIS NO.: 000034012581
ISOLATION OF THE LIPOPROTEIN ASSOCIATED COAGULATION
  INHIBITOR PRODUCED BY HEPG2 HUMAN HEPATOMA CELLS USING BOVINE
  FACTOR XA AFFINITY CHROMATOGRAPHY
AUTHOR: BROZE G J JR; WARREN L A; GIRARD J J; MILETICH J P
AUTHOR ADDRESS: DIV. HEMATOL./ONCOL., WASHINGTON UNIV. SCH. MED., JEWISH
  HOSP., ST. LOUIS, MO., USA.
JOURNAL: THROMB RES 48 (2). 1987. 253-260. 1987
FULL JOURNAL NAME: Thrombosis Research
CODEN: THBRA
RECORD TYPE: Citation
LANGUAGE: ENGLISH


 7/7/2     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

05747045   88100467   PMID: 2827342
    Isolation of the lipoprotein associated coagulation
inhibitor produced by HepG2 (human hepatoma) cells using bovine
factor Xa affinity chromatography.
  Broze G J; Warren L A; Girard J J; Miletich J P
  Division of Hematology/Oncology, Washington University School of
Medicine, Jewish Hospital, St. Louis, Missouri.
  Thrombosis research (UNITED STATES) Oct 15 1987, 48 (2) p253-9
,  ISSN 0049-3848  Journal Code: 0326377
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed
  Record Date Created: 19880127
  Record Date Completed: 19880127


 7/7/3     (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

05496772   87175579   PMID: 3031657
  Isolation of the tissue factor inhibitor produced by HepG2 hepatoma
cells.
  Broze G J; Miletich J P
  Proceedings of the National Academy of Sciences of the United States of
America (UNITED STATES) Apr 1987, 84 (7) p1886-90, ISSN
0027-8424  Journal Code: 7505876
  Contract/Grant No.: HL34462; HL; NHLBI
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed
```

Progressive inhibition of tissue factor activity occurs upon its addition to human plasma (serum). This process requires the presence of factor VII(a), facto-X(a), Ca2+, and another component in plasma that we have called the tissue factor inhibitor (TFI). A TFI secreted by HepG2 cells (human hepatoma cell line) was isolated from serum-free conditioned medium in a four-step procedure including CdCl2 precipitation, diisopropylphosphoryl-factor Xa affinity chromatography, Sephadex G-75 superfine gel filtration, and Mono Q ion-exchange chromatography. The purified TFI contained a predominant band at Mr 38,000 on NaDodSO4/polyacrylamide gel electrophoresis that comigrates with inhibitory activity. Like the activity present in plasma, this TFI requires the presence of factor VII(a), factor X(a), and Ca2+ to express inhibitory activity. Its specific activity (assuming an extinction coefficient of 10 at 280 nM, for a 1-cm path length through a 1% solution) was 9800 units/mg of protein, where 1 unit of TFI activity was defined as that present in 1 ml of normal pooled serum.
Record Date Created: 19870504
Record Date Completed: 19870504

7/7/4 (Item 1 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

112135018 CA: 112(15)135018p JOURNAL
Isolation of the lipoprotein associated coagulation inhibitor produced by HepG2 (human hepatoma) cells using bovine factor Xa affinity chromatography
AUTHOR(S): Broze, George J., Jr.; Warren, Louise A.; Girard, James J.; Miletich, Joseph P.
LOCATION: Sch. Med., Washington Univ., St. Louis, MO, USA
JOURNAL: Thromb. Res. DATE: 1987 VOLUME: 48 NUMBER: 2 PAGES: 253-9
CODEN: THBRAA ISSN: 0049-3848 LANGUAGE: English
SECTION:
CA207003 Enzymes
CA213XXX Mammalian Biochemistry
IDENTIFIERS: lipoprotein assocd coagulation inhibitor affinity chromatog, blood lipoprotein assocd coagulation inhibitor purifn, protein inhibitor lipoprotein assocd coagulation purifn
DESCRIPTORS:
Blood-coagulation factors,LACI (lipoprotein-assocd. coagulation inhibitor)
...
of HepG2 cells, of human, affinity chromatog. in isolation of
? ds

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Set     Items   Description
S1          1   AU='O BRIEN D P'
S2       4094   (LACI OR LIPOPROTEIN(W)ASSOCIATED(W)COAGULATION)
S3        517   S2 AND PY<1989
S4       1030   S2 AND COAGULATION
S5       1025   S4 AND LIPOPROTEIN
S6       1028   S2 AND COAGULATION(20N)(TREAT? OR INHIBIT? OR SUPPRESS? OR
            THERAP? OR BLOCK? OR ANTAGONI?)
S7          4   S6 AND PY=1987
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           1028  S6
        1645762  PY=1988
      S8      17  S6 AND PY=1988
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...completed examining records
      S9       8  RD S8 (unique items)
? t s9/7/all
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9/7/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

06602335 BIOSIS NO.: 000087044497
PLATELETS SECRETE A **COAGULATION INHIBITOR** FUNCTIONALLY AND ANTIGENICALLY SIMILAR TO THE **LIPOPROTEIN ASSOCIATED COAGULATION INHIBITOR**
AUTHOR: NOVOTNY W F; GIRARD T J; MILETICH J P; BROZE G J JR
AUTHOR ADDRESS: JEWISH HOSPITAL/HEMATOL. RES., 216 S KINGSHIGHWAY, ST. LOUIS, MO 63110.
JOURNAL: BLOOD 72 (6). 1988. 2020-2025. **1988**
FULL JOURNAL NAME: Blood
CODEN: BLOOA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Stimulation with thrombin or the calcium ionophore, A23187 caused human platelets to release a **coagulation inhibitor** similar to the **Lipoprotein Associated Coagulation Inhibitor** (LACl). This was documented functionally, with clotting assays measuring tissue factor **inhibition** and factor Xa **inhibition**, as well as immunologically, in a competitive immunoassay. The total amount of LACl released by 3 .times. 108 plateletes after two hours stimulation was 7% to 8% of the amount found in 1 mL of serum. Half of the LACl was released by five minutes. The LACl was present in the platelet supernatant and was not associated with the platelet membrane or shed vesicles. The tissue factor and factor Xa inhibitory activities that were released were neutralized by preincubating the platelet supernatants with specific rabbit polyclonal anti-LACl IgG. On Western blot, platelet LACl appeared to run as a doublet with a molecular weight (mol wt) 45,000 to 47,000. Blood samples obtained from the site of a wound (template bleeding time) demonstrated a progressive increase in LACl concentration. A cDNA probe, derived from endothelial cell LACl cDNA, hybridized selectively to 4.0 and 1.4 kb transcripts in a preparation of platelet mRNA.

9/7/2 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

06371683 BIOSIS NO.: 000036074836
HUMAN FIBROBLAST TISSUE FACTOR IS **INHIBITED** BY **LIPOPROTEIN-ASSOCIATED COAGULATION INHIBITOR** AND PLACENTAL ANTICOAGULANT PROTEIN BUT NOT BY APOLIPOPROTEIN A-II
AUTHOR: GRAMZINSKI R A; NOVOTNY W F; BROZE G J JR; CARSON S D
AUTHOR ADDRESS: UNIV. COLO. SCH. MED., DENVER, COLO.
JOURNAL: 8TH NATIONAL CONFERENCE ON THROMBOSIS AND HEMOSTASIS, WASHINGTON, D.C., USA, NOVEMBER 1988. ARTERIOSCLEROSIS 8 (5). 1988. 680A. **1988**
CODEN: ARTRD
DOCUMENT TYPE: Meeting
RECORD TYPE: Citation
LANGUAGE: ENGLISH

9/7/3 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

06180909 BIOSIS NO.: 000086015091
CLONING AND CHARACTERIZATION OF A COMPLEMENTARY DNA CODING FOR THE **LIPOPROTEIN**-ASSOCIATED **COAGULATION INHIBITOR** SHOWS THAT IT CONSISTS OF THREE TANDEM KUNITZ-TYPE **INHIBITORY** DOMAINS
AUTHOR: WUN T-C; KRETZMER K K; GIRARD T J; MILETICH J P; BROZE G J JR
AUTHOR ADDRESS: MONSANTO CO., CHESTERFIELD, MO. 63198.
JOURNAL: J BIOL CHEM 263 (13). 1988. 6001-6004. **1988**

FULL JOURNAL NAME: Journal of Biological Chemistry
CODEN: JBCHA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Human plasma contains a **lipoprotein**-associated **coagulation inhibitor** (**LACI**) which inactivates factor Xa directly, and in a Xa-dependent fashion also **inhibits** the VIIa-tissue factor complex of the extrinsic **coagulation** pathway. Rabbit polyclonal anti-**LACI** antiserum was used to screen human placental and fetal liver .lambda.gt11 cDNA libraries for the expression of **LACI** antigens. Immunologically positive clones were further tested for their ability to bind 125I-factor Xa. Seven clones were obtained which are immunologically and functionally active. The longest cDNA insert (.lambda.P9) of these isolates is 1.4 kilobases (kb) while other clones are 1.0 kb in length. Nucleotide sequence analysis shows that .lambda.P9 consists of 1431 bases that include a 5'-noncoding sequence of 132 nucleotides, an open reading frame of 912 nucleotides, and a 3'-noncoding region of 387 nucleotides. The open reading frame encodes a signal peptide of 28 residues followed by a 32-kilodalton protein of 276 residues. The predicted sequence of mature **LACI** contains 18 cysteines and three potential N-linked glycosylation sites. The amino acid sequence analysis of purified **LACI**'s NH2 terminus and two of its proteolytic fragments match exactly those deduced from the cDNA sequence, indicating that the cDNA codes for **LACI**. The translated amino acid sequence of **LACI** shows several discernible domains, including a highly negatively charged NH2 terminus, three tandem Kunitz-type inhibitory domains, and a highly positively charged carboxyl terminus. Northern blot analysis shows that the following liver-derived cell lines, Chang liver, HepG2 hepatoma, and SK hepatoma all, contain two major species of mRNA (1.4 and 4.4 kb) which hybridize with **LACI** cDNA.

9/7/4 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

06144568 BIOSIS NO.: 000085107720
THE **LIPOPROTEIN**-ASSOCIATED **COAGULATION INHIBITOR** THAT **INHIBITS** THE FACTOR-VII-TISSUE FACTOR COMPLEX ALSO **INHIBITS** FACTOR XA INSIGHT INTO ITS POSSIBLE MECHANISM OF ACTION
AUTHOR: BROZE G J JR; WARREN L A; NOVOTNY W F; HIGUCHI D A; GIRARD J J; MILETICH J P
AUTHOR ADDRESS: JEWISH HOSP., 216 S. KINGSHIGHWAY, ST. LOUIS, MO. 63110.
JOURNAL: BLOOD 71 (2). 1988. 335-343. **1988**
FULL JOURNAL NAME: Blood
CODEN: BLOOA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Blood coagulation is initiated when plasma factor VII(a) binds to its essential cofactor tissue factor (TF) and proteolytically activates factors X and IX. Progressive inhibition of TF activity occurs upon its addition to plasma. This process is reversible and requires the presence of VII(a), catalytically active Xa, Ca2+, and another component that appears to be associated with the lipoproteins in plasma, a **lipoprotein**-associated **coagulation inhibitor** (**LACI**). A protein, **LACI**(HG2), possessing the same **inhibitory** properties as **LACI**, has recently been isolated from the conditioned media of cultured human liver cells (HepG2). Rabbit antisera raised against a synthetic peptide based on the N-terminal sequence of **LACI**(HG2) and purified IgG from a rabbit immunized with intact **LACI**(HG2) inhibit the **LACI** activity in human serum. In

a reaction mixture containing VII, Xa, Ca2+, and purified **LACI** (HG2), the apparent half-life (t1/2) for TF activity was 20 seconds. The presence of heparin accelerated the initial rate of inhibition threefold. Antithrombin III.alpha. alone had no effect, but antithrombin III.alpha. with heparin abrogated the TF inhibition. **LACI**(HG2) also inhibited Xa with an apparent t1/2 of 50 seconds. Heparin enhanced the rate of Xa inhibition 2.5-fold, whereas phospholipids and Ca2+ slowed the reaction 2.5-fold. Xa inhibition was demonstrable with both chromogenic substrate (S-222) and bioassays, but no complex between Xa and LAC(HG2) could be visualized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Nondenaturing PAGE, however, showed that **LACI**(HG2) bound to Xa but not to X or Xa inactivated by diisopropyl fluorophosphate. Thus, **LACI**(HG2) appears to bind to Xa at or near its active site. Bovine factor Xa lacking its .gamma.-carboxyglutamic acid-containing domain, BXa(-GD), through treatment with .alpha.-chymotrypsin, was used to further investigate the Xa requirement for VIIa/TF inhibition by **LACI**(HG2). **LACI**(HG2) bound to BXa(-GD) and inhibited its catalytic activity against a small molecular substrate (Spectrozyme Xa), though at a rate approximately sevenfold slower than native BXa. Preincubation of **LACI**(HG2) with saturating concentrations of BXa(-GD) markedly retarded the subsequent inhibition of BXa. The VII(a)/TF complex was not inhibited by **LACI**(HG2) in the presence of BXa(-GD), and further, preincubation of **LACI**(HG2) with BXa(-GD) slowed the inhibition of VIIa/TF after the addition of native Xa. The results are consistent with the hypothesis that inhibition of VII(a)/TF involves the formation of a VIIa-TF-Xa-**LACI** complex that requires the GD of Xa. Because the GD contains the .alpha.-carboxyglutamic acids required for the Ca2+-dependent binding of factor Xa to phospholipid surfaces, the results also suggest that Ca2+ may be required for the native Xa-**LACI** complex to bind to and inhibit VII(A)/TF. **LACI** is a novel inhibitor that can rapidly affect feedback inhibition of the VIIa-Ca2+-TF enzymatic complex after the generation of small amounts of Xa and probably plays an important role in the regulation of in vivo coagulation.

9/7/5 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

05968744 BIOSIS NO.: 000035060107
PLATELETS RELEASE THE **LIPOPROTEIN** ASSOCIATED **COAGULATION INHIBITOR** UPON STIMULATION WITH THROMBIN OR A-23187
AUTHOR: NOVOTNY W F; MILETICH J P; BROZE G J JR
AUTHOR ADDRESS: JEWISH HOSP. AT WASHINGTON UNIV. MED. CENT., ST. LOUIS, MO.
JOURNAL: EIGHTIETH ANNUAL NATIONAL MEETING OF THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION, WASHINGTON, D.C., USA, APRIL 29-MAY 2, 1988. CLIN RES 36 (3). 1988. 568A. **1988**
CODEN: CLREA
DOCUMENT TYPE: Meeting
RECORD TYPE: Citation
LANGUAGE: ENGLISH

9/7/6 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

05968728 BIOSIS NO.: 000035060091
CLONING AND SEQUENCING OF A COMPLEMENTARY DNA FOR THE HUMAN **LIPOPROTEIN** ASSOCIATED **COAGULATION INHIBITOR**
AUTHOR: GIRARD T J; WARREN L A; NOVOTNY W F; MILETICH J P; BROZE G J JR
AUTHOR ADDRESS: JEWISH HOSP. AT WASHININGTON UNIV. MED. CENT., ST. LOUIS, MO.

JOURNAL: EIGHTIETH ANNUAL NATIONAL MEETING OF THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION, WASHINGTON, D.C., USA, APRIL 29-MAY 2, 1988. CLIN RES 36 (3). 1988. 565A. **1988**
CODEN: CLREA
DOCUMENT TYPE: Meeting
RECORD TYPE: Citation
LANGUAGE: ENGLISH

03640083 EMBASE No: 1988089519

The **lipoprotein**-associated **coagulation inhibitor** that **inhibits** the factor VII-tissue factor complex also **inhibits** factor Xa: Insight into its possible mechanism of action

Broze Jr. G.J.; Warren L.A.; Novotny W.F.; Higuchi D.A.; Girard J.J.; Miletich J.P.
Jewish Hospital, St Louis, MO 63110 United States

Blood (BLOOD) (United States) 1988, 71/2 (335-343)
CODEN: BLOOA ISSN: 0006-4971
DOCUMENT TYPE: Journal
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

Blood coagulation is initiated when plasma factor VII(a) binds to its essential cofactor tissue factor (TF) and proteolytically activates factors X and IX. Progressive inhibition of TF activity occurs upon its addition to plasma. This process is reversible and requires the presence of VII(a), catalytically active Xa, Casup 2sup +, and another component that appears to be associated with the lipoproteins in plasma, a **lipoprotein** -associated **coagulation inhibitor** (**LACI**). A protein, **LACI**(HG2), possessing the same **inhibitory** properties as **LACI**, has recently been isolated from the conditioned media of cultured human liver cells (HepG2). Rabbit antisera raised against a synthetic peptide based on the N-terminal sequence of **LACI**(HG2) and purified IgG from a rabbit immunized with intact **LACI**(HG2) inhibit the **LACI** activity in human serum. In a reaction mixture containing VIIa, Xa, Casup 2sup +, and purified **LACI**(HG2), the apparent half-life (t1/2) for TF activity was 20 seconds. The presence of heparin accelerated the initial rate of inhibition threefold. Antithrombin IIIalpha alone had no effect, but antithrombin IIIalpha with heparin abrogated the TF inhibition. **LACI**(HG2) also inhibited Xa with an apparent t1/2 of 50 seconds. Heparin enhanced the rate of Xa inhibition 2.5-fold, whereas phospholipids and Casup 2sup + slowed the reaction 2.5-fold. Xa inhibition was demonstrable with both chromogenic substrate (S-2222) and bioassays, but no complex between Xa and **LACI**(HG2) could be visualized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Nondenaturing PAGE, however, showed that **LACI**(HG2) bound to Xa but not to X or Xa inactivated by diisopropyl fluorophosphate. Thus, **LACI** (HG2) appears to bind to Xa at or near its active site. Bovine factor Xa lacking its gamma-carboxyglutamic acid-containing domain, BXa(-GD), through treatment with alpha-chymotrypsin, was used to further investigate the Xa requirement for VIIa/TF inhibition by **LACI**(HG2). **LACI**(HG2) bound to BXa(-GD) and inhibited its catalytic activity against a small molecular substrate (Spectrozyme Xa), though at a rate approximately sevenfold slower than native BXa. Preincubation of **LACI**(HG2) with saturating concentrations of BXa(-GD) markedly retarded the subsequent inhibition of BXa. The VII(a)/TF complex was not inhibited by **LACI** (HG2) in the presence of BXa(-GD), and further, preincubation of **LACI** (HG2) with BXa(-GD) slowed the inhibition of VIIa/TF after the addition of native Xa. The results are consistent with the hypothesis that inhibiton of VII(a)/TF involves the formation of a VIIa-TF-Xa-**LACI** complex that requires the GD of Xa. Because the GD contains the alpha-carboxyglutamic

acids required for the Casup 2sup +-dependent binding of factor Xa to phospholipid surfaces, the results also suggest that Casup 2sup + may be required for the native Xa-**LACI** complex to bind to and inhibit VII(a)/TF. **LACI** is a novel inhibitor that can rapidly affect feedback inhibition of the VIIa-Casup 2sup +-TF enzymatic complex after the generation of small amounts of Xa and probably plays an important role in the regulation of the in vivo coagulation.

9/7/8 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06172204 89187705 PMID: 3238648
Modifications of extrinsic pathway inhibitor (EPI) and factor Xa that affect their ability to interact and to inhibit factor VIIa/tissue factor: evidence for a two-step model of inhibition.
Warn-Cramer B J; Rao L V; Maki S L; Rapaport S I
Department of Medicine, University of California, San Diego, La Jolla 92103.
Thrombosis and haemostasis (GERMANY, WEST) Dec 22 **1988**, 60 (3) p453-6, ISSN 0340-6245 Journal Code: 7608063
Contract/Grant No.: H L27234; PHS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Inhibition of factor VIIa/tissue factor (TF) by extrinsic pathway inhibitor (EPI) requires the participation of factor Xa. Through this inhibition, factor Xa generated initially may feed back to suppress continuing generation of factor Xa via the extrinsic pathway during hemostasis. We have utilized chemical modifications of EPI and factor Xa to study the reactions responsible for inhibition. The data are consistent with a two-step model. First, EPI binds to factor Xa in a Ca2+ independent reaction in which the gla-domain of factor Xa does not participate. A functional active site on factor Xa and arginine residues on EPI are essential for this step. Then the factor Xa/EPI complex binds to factor VIIa/TF with resultant inhibition of its enzymatic activity. The gla-domain of factor Xa is essential for this step. Intact positively charged lysines on factor Xa may also be important.
Record Date Created: 19890501
Record Date Completed: 19890501
?

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TEM:OS  - DIALOG OneSearch
  File   5:Biosis Previews(R)  1969-2003/Jul W4
         (c) 2003 BIOSIS
  File  73:EMBASE  1974-2003/Jul W4
         (c) 2003 Elsevier Science B.V.
*File  73: Alert feature enhanced for multiple files, duplicates
removal, customized scheduling. See HELP ALERT.
  File 155:MEDLINE(R)  1966-2003/Aug W1
         (c) format only 2003 The Dialog Corp.
*File 155: Medline has been reloaded and accession numbers have
changed.  Please see HELP NEWS 155.
  File 399:CA SEARCH(R)  1967-2003/UD=13906
         (c) 2003 American Chemical Society
*File 399: Use is subject to the terms of your user/customer agreement.
Alert feature enhanced for multiple files, etc. See HELP ALERT.

      Set  Items  Description
      ---  -----  -----------
? s (tgf? or transforming(W)growth(w)factor) and (ARDS or
adult(W)respiratory(W)distress)
Processing
          81142  TGF?
         156004  TRANSFORMING
        2539910  GROWTH
        2299383  FACTOR
         106420  TRANSFORMING(W)GROWTH(W)FACTOR
          10441  ARDS
        4624067  ADULT
         922801  RESPIRATORY
         113855  DISTRESS
          16604  ADULT(W)RESPIRATORY(W)DISTRESS
      S1     60  (TGF? OR TRANSFORMING(W)GROWTH(W)FACTOR) AND (ARDS OR
                 ADULT(W)RESPIRATORY(W)DISTRESS)
? rd s1
...examined 50 records  (50)
...completed examining records
      S2     45  RD S1 (unique items)
? t s2/3/all

 2/3/1     (Item 1 from file: 5)
DIALOG(R)File   5:Biosis Previews(R)
(c) 2003 BIOSIS. All rts. reserv.

14240009   BIOSIS NO.: 200300234038
Mechanisms of alcohol-induced tissue injury.
AUTHOR: Molina Patricia E(a); Hoek Jan B; Nelson Steve; Guidot David M;
  Lang Charles H; Wands Jack R; Crawford James M
AUTHOR ADDRESS: (a)Department of Physiology, LSUHSC, 1901 Perdido Street,
  New Orleans, LA, 70112, USA**USA E-Mail: pmolin@lsuhsc.edu
JOURNAL: Alcoholism Clinical and Experimental Research  27 (3):p563-575
March 2003 2003
MEDIUM: print
ISSN: 0145-6008
DOCUMENT TYPE: Article
RECORD TYPE: Citation
LANGUAGE: English


 2/3/2     (Item 2 from file: 5)
DIALOG(R)File   5:Biosis Previews(R)
(c) 2003 BIOSIS. All rts. reserv.

14231352   BIOSIS NO.: 200300225381
The acute respiratory distress syndrome: A role for transforming
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growth factor-beta1.
AUTHOR: Fahy Ruairi J(a); Lichtenberger Frank; McKeegan Christine B; Nuovo Gerard J; Marsh Clay B; Wewers Mark D
AUTHOR ADDRESS: (a)The Davis Heart and Lung Research Institute, 473 West 12th Avenue, 201, Columbus, OH, 43210-1252, USA**USA E-Mail: fahy-1@medctr.osu.edu
JOURNAL: American Journal of Respiratory Cell and Molecular Biology 28 (4):p499-503 April 2003 2003
MEDIUM: print
ISSN: 1044-1549
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

2/3/3 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

14117115 BIOSIS NO.: 200300111144
Roles for insulin-like growth factor I and **transforming growth factor**-beta in fibrotic lung disease.
AUTHOR: Krein Peter M; Winston Brent W(a)
AUTHOR ADDRESS: (a)Departments of Medicine, Biochemistry and Molecular Biology, and Critical Care Medicine, Health Sciences Center, University of Calgary, 3330 Hospital Dr NW, Room 1843, Calgary, AB, T2N 4N1, Canada **Canada E-Mail: bwinston@ucalgary.ca
JOURNAL: Chest 122 (6 Suppl.):p289S-293S December 2002 2002
MEDIUM: print
ISSN: 0012-3692
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

2/3/4 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13887476 BIOSIS NO.: 200200516297
Tumor necrosis factor-alpha and angiostatin are mediators of endothelial cytotoxicity in bronchoalveolar lavages of patients with acute respiratory distress syndrome.
AUTHOR: Hamacher Juerg; Lucas Rudolf(a); Lijnen H Roger; Buschke Susanne; Dunant Yves; Wendel Albrecht; Grau Georges E; Suter Peter M; Ricou Bara
AUTHOR ADDRESS: (a)Biochemical Pharmacology, Department of Biology, Universitaetstrasse 10, D-78457, Box M668, Konstanz**Germany E-Mail: rudolf.lucas@uni-konstanz.de
JOURNAL: American Journal of Respiratory and Critical Care Medicine 166 (5):p651-656 September 1, 2002
MEDIUM: print
ISSN: 1073-449X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

2/3/5 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13845032 BIOSIS NO.: 200200473853
TGF-beta alters steroid-induced expression of sodium channels in distal lung epithelia.

AUTHOR: Jain Lucky(a); Chen Xi-Juan(a); Eaton Douglas C(a)
AUTHOR ADDRESS: (a)Pediatrics and Physiology, Emory University, Atlanta, GA **USA
JOURNAL: Pediatric Research 51 (4 Part 2):p474A April, 2002
MEDIUM: print
CONFERENCE/MEETING: Annual Meeting of the Pediatric Societies' Baltimore, MD, USA May 04-07, 2002
ISSN: 0031-3998
RECORD TYPE: Citation
LANGUAGE: English

2/3/6 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13819010 BIOSIS NO.: 200200447831
Metalloproteinase and growth factor interactions: Do they play a role in pulmonary fibrosis?
AUTHOR: Winkler Margaret K(a); Fowlkes John L
AUTHOR ADDRESS: (a)1600 7th Ave. S., ACC No. 504, Birmingham, AL, 35233** USA E-Mail: Mwinkler@peds.uab.edu
JOURNAL: American Journal of Physiology 283 (1 Part 1):pL1-L11 July, 2002
MEDIUM: print
ISSN: 0002-9513
DOCUMENT TYPE: Literature Review
RECORD TYPE: Abstract
LANGUAGE: English

2/3/7 (Item 7 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13160861 BIOSIS NO.: 200100368010
TNF and angiostatin but not **TGF**-beta1 are mediators endothelial cells in bronochoalveolar lavages of **ARDS** patients.
AUTHOR: Hamacher J(a); Ricou B; Dunant Y; Lijnen H R; Grau G E; Wendel A(a); Suter P M; Lucas R(a)
AUTHOR ADDRESS: (a)Biochemical Pharmacology, University of Konstanz, D-78457, Konstanz**Germany
JOURNAL: Naunyn-Schmiedeberg's Archives of Pharmacology 363 (4 Supplement):pR110 2001
MEDIUM: print
CONFERENCE/MEETING: 42nd Spring Meeting of the German Society for Experimental and Cllinical Pharmacology and Toxicology Mainz, Germany March 13-15, 2001
ISSN: 0028-1298
RECORD TYPE: Citation
LANGUAGE: English
SUMMARY LANGUAGE: English

2/3/8 (Item 8 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13123403 BIOSIS NO.: 200100330552
Aerosolized perfluorocarbon decreases pulmonary inflammatory response and improves gas exchange in piglets with **ARDS**.
AUTHOR: von der Hardt Katharina(a); Kandler Michael A(a); Schoof Ellen(a); Doetsch Joerg(a); Rascher Wolfgang(a)
AUTHOR ADDRESS: (a)Klinik fuer Kinder und Jugendliche, Universitaet Erlangen-Nuernberg, Erlangen**Germany

JOURNAL: Pediatric Research 49 (4 Part 2):p46A April, 2001
MEDIUM: print
CONFERENCE/MEETING: Annual Meeting of the Pediatric Academic Societies Baltimore, Maryland, USA April 28-May 01, 2001
ISSN: 0031-3998
RECORD TYPE: Citation
LANGUAGE: English
SUMMARY LANGUAGE: English

2/3/9 (Item 9 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13026174 BIOSIS NO.: 200100233323
Osteopontin: A key cytokine in cell-mediated and granulomatous inflammation.
AUTHOR: O'Regan Anthony; Berman Jeffrey S(a)
AUTHOR ADDRESS: (a)Pulmonary Center, Boston University School of Medicine, R304, Boston, MA, 02118: jberman@lung.bumc.bu.edu**USA
JOURNAL: International Journal of Experimental Pathology 81 (6):p373-390 December, 2000
MEDIUM: print
ISSN: 0959-9673
DOCUMENT TYPE: Literature Review
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

2/3/10 (Item 10 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11658919 BIOSIS NO.: 199800440650
Elevated **transforming growth factor**-alpha levels in bronchoalveolar lavage fluid of patients with acute respiratory distress syndrome.
AUTHOR: Madtes David K(a); Rubenfeld Gordon; Klima Lawrence D; Milberg John A; Steinberg Kenneth P; Martin Thomas R; Raghu Ganesh; Hudson Leonard D; Clark Joan G
AUTHOR ADDRESS: (a)Fred Hutchinson Cancer Res. Center, 1124 Columbia St. M677, Seattle, WA 98104-2092**USA
JOURNAL: American Journal of Respiratory and Critical Care Medicine 158 (2):p424-430 Aug., 1998
ISSN: 1073-449X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

2/3/11 (Item 11 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11399532 BIOSIS NO.: 199800180864
Hyperoxia influences mRNA expression of cytokines in cultured human umbilical vein endothelial cells.
AUTHOR: Park Min Soo(a); Wallace Heather M
AUTHOR ADDRESS: (a)Dep. Pediatr., Yongdong Severance Hosp., Yonsei Univ. Coll. Med., Yongdong PO Box 1217, Seoul 13**South Korea
JOURNAL: Yonsei Medical Journal 39 (1):p1-12 Feb., 1998
ISSN: 0513-5796
DOCUMENT TYPE: Article

RECORD TYPE: Abstract
LANGUAGE: English

2/3/12 (Item 12 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11361498 BIOSIS NO.: 199800142830
Upregulation of postbacteremic TNF-alpha and IL-1alpha gene expression by alveolar hypoxia/reoxygenation in perfused rat lungs.
AUTHOR: Matuschak George M(a); Munoz Cesar F; Johanns Cheryl A; Rahman Rashid; Lechner Andrew J
AUTHOR ADDRESS: (a)Div. Pulmonol., Saint Louis Univ. Hosp., 3635 Vista Ave. at Grand Blvd., Saint Louis, MO 63110-0**USA
JOURNAL: American Journal of Respiratory and Critical Care Medicine 157 (2):p629-637 Feb., 1998
ISSN: 1073-449X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

2/3/13 (Item 13 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10800967 BIOSIS NO.: 199799422112
Platelet-derived growth factors and tissue repair.
AUTHOR: Vaillant P
AUTHOR ADDRESS: Fed. Med.-Chir. Pneumol., CHU Nancy, 54511 Vandoeuvre** France
JOURNAL: Revue des Maladies Respiratoires 13 (6):p555-558 1996
ISSN: 0761-8425
RECORD TYPE: Citation
LANGUAGE: French; Non-English

2/3/14 (Item 14 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10522174 BIOSIS NO.: 199699143319
Apoptosis in human alveolar macrophages is induced by endotoxin and is modulated by cytokines.
AUTHOR: Bingisser Roland; Stey Claudia; Weller Michael; Groscurth Peter; Russi Erich; Frei Karl
AUTHOR ADDRESS: Universitatsspital, 8091 Zurich**Switzerland
JOURNAL: American Journal of Respiratory Cell and Molecular Biology 15 (1):p64-70 1996
ISSN: 1044-1549
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

2/3/15 (Item 15 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

09504534 BIOSIS NO.: 199497512904
Anti-**transforming growth factor**-beta monoclonal antibodies prevent lung injury in hemorrhaged mice.
AUTHOR: Shenkar Robert; Coulson Walter F; Abraham Edward(a)

AUTHOR ADDRESS: (a)Div. Pulmonary Sci. and Critical Care Med., Univ. Colo. Health Sci. Cent., Box C272, 4200 E. Nin**USA
JOURNAL: American Journal of Respiratory Cell and Molecular Biology 11 (3):p351-357 1994
ISSN: 1044-1549
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

2/3/16 (Item 16 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

09433654 BIOSIS NO.: 199497442024
Elevated **TGF**-beta activity in patients with severe **ARDS** on extracorporeal life support.
AUTHOR: Fazzalari Franco(a); Phan Shem; Bonnell Mark; Bliss David; Hirschl Ron; Bartlett Robert
AUTHOR ADDRESS: (a)Dep. Surg., Univ. Mich. Med. Sch., Ann Arbor, MI 48109** USA
JOURNAL: Chest 106 (2 SUPPL.):p64S 1994
CONFERENCE/MEETING: 60th Annual Scientific Assembly of the American College of Chest Physicians New Orleans, Louisiana, USA October 30-November 3, 1994
ISSN: 0012-3692
RECORD TYPE: Citation
LANGUAGE: English

2/3/17 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

12178947 EMBASE No: 2003291807
Cell biology and the respiratory system
BIOLOGIE CELLULAIRE ET SYSTEME RESPIRATOIRE
Fellrath J.-M.; Kantengwa S.; Nicod L.-P.
J.-M. Fellrath, Division de Pneumologie, Hop. Cant. Universitaire de Geneve, CH-1211 Geneve 14 Switzerland
Revue des Maladies Respiratoires (REV. MAL. RESPIR.) (France) 2003, 20/SPEC. (5S10-5S19)
CODEN: RMREE ISSN: 0761-8425
DOCUMENT TYPE: Journal ; Review
LANGUAGE: FRENCH

2/3/18 (Item 2 from file: 73)
DIALOG(R)File 73:EMBASE

12177073 EMBASE No: 2003288818
Lung injury after thoracotomy
Baudouin S.V.
S.V. Baudouin, Department of Anaesthesia, Royal Victoria Infirmary, Leazes Wing, Newcastle Upon Tyne United Kingdom
AUTHOR EMAIL: S.V.Baudouin@ncl.ac.uk
British Journal of Anaesthesia (BR. J. ANAESTH.) (United Kingdom) 01 JUL 2003, 91/1 (132-142)
CODEN: BJANA ISSN: 0007-0912
DOCUMENT TYPE: Journal ; Review
LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 113

2/3/19 (Item 3 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2003 Elsevier Science B.V. All rts. reserv.

12053275 EMBASE No: 2003163781
Transforming growth factor-beta: A mediator of cell regulation in acute respiratory distress syndrome
Dhainaut J.-F.; Charpentier J.; Chiche J.-D.
Dr. J.-F. Dhainaut, Faculte Cochin Port-Royal, Universite Paris 5, Hopital Cochin, Paris Cedex 14 France
Critical Care Medicine (CRIT. CARE MED.) (United States) 01 APR 2003 , 31/4 SUPPL. (S258-S264)
CODEN: CCMDC ISSN: 0090-3493
DOCUMENT TYPE: Journal ; Conference Paper
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 70

2/3/20 (Item 4 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2003 Elsevier Science B.V. All rts. reserv.

11897587 EMBASE No: 2003009003
Acute respiratory distress syndrome: Fibrosis fast and furious
Chua F.; Bellingan G.J.
G.J. Bellingan, Centre for Respiratory Research, University College London Hospitals, The Rayne Institute, 5 University Street, London United Kingdom
AUTHOR EMAIL: g.bellingan@ucl.ac.uk
Clinical Intensive Care (CLIN. INTENSIVE CARE) (United Kingdom) 2002 , 13/2-3 (65-72)
CODEN: CICAE ISSN: 0956-3075
DOCUMENT TYPE: Journal ; Review
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 71

2/3/21 (Item 5 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2003 Elsevier Science B.V. All rts. reserv.

11852060 EMBASE No: 2002426537
Talc for pleurodesis?
Light R.W.
Dr. R.W. Light, Dept. of Pulmonary Disease Program, Saint Thomas Hospital, 4220 Harding Rd, Nashville, TN United States
AUTHOR EMAIL: RLIGHT98@yahoo.com
Chest (CHEST) (United States) 2002, 122/5 (1506-1508)
CODEN: CHETB ISSN: 0012-3692
DOCUMENT TYPE: Journal ; Editorial
LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 25

2/3/22 (Item 6 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2003 Elsevier Science B.V. All rts. reserv.

11677698 EMBASE No: 2002243027
Acute respiratory distress syndrome epidemiology and pathophysiology
Morrison R.J.; Bidani A.
Dr. A. Bidani, Division of Pulmonary Medicine, Baylor College of Medicine, 6550 Fannin, Houston, TX 77030 United States

AUTHOR EMAIL: abidani@bcm.tmc.edu
Chest Surgery Clinics of North America (CHEST SURG. CLIN. NORTH AM.) (United States) 2002, 12/2 (301-323)
CODEN: CSCAF ISSN: 1052-3359
PUBLISHER ITEM IDENTIFIER: S1052335902000042
DOCUMENT TYPE: Journal ; Review
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 105

2/3/23 (Item 7 from file: 73)
DIALOG(R)File 73:EMBASE

11640168 EMBASE No: 2002212502
The pulmonary physician in critical care.6: The pathogenesis of ALI/**ARDS**
Bellingan G.J.
G.J. Bellingan, University College Hospitals, Centre for Respiratory Research, Rayne Institute, London WC1E 6JJ United Kingdom
Thorax (THORAX) (United Kingdom) 2002, 57/6 (540-546)
CODEN: THORA ISSN: 0040-6376
DOCUMENT TYPE: Journal ; Review
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 92

2/3/24 (Item 8 from file: 73)
DIALOG(R)File 73:EMBASE

11491892 EMBASE No: 2002063529
Inflammation
Cone J.B.
Dr. J.B. Cone, Department of Surgery, Mail Slot 520-1, Univ. Arkansas for Medical Sciences, 4301 W. Markham, Little Rock, AR 72205 United States
AUTHOR EMAIL: conejohnb@uams.edu
American Journal of Surgery (AM. J. SURG.) (United States) 2001, 182/6 (558-562)
CODEN: AJSUA ISSN: 0002-9610
PUBLISHER ITEM IDENTIFIER: S0002961001008224
DOCUMENT TYPE: Journal ; Conference Paper
LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 12

2/3/25 (Item 9 from file: 73)
DIALOG(R)File 73:EMBASE

10983253 EMBASE No: 2001025465
What are the markers for pulmonary fibrosis in acute respiratory distress syndrome?
QUELS SONT LES MARQUEURS DE LA FIBROSE PULMONAIRE AU COURS DU SYNDROME DE DETRESSE RESPIRATOIRE AIGUE?
Delclaux C.
C. Delclaux, Service de Physiologie, Hopital Henri-Mondor, 51, avenue du M.-de-Lattre-Tassigny, 94010 Creteil France
AUTHOR EMAIL: delclaux@im3.inserm.fr
Reanimation Urgences (REANIM. URGENCES) (France) 2000, 9/8 (628-632)
CODEN: REURF ISSN: 1164-6756
DOCUMENT TYPE: Journal ; Conference Paper
LANGUAGE: FRENCH
NUMBER OF REFERENCES: 28

2/3/26 (Item 10 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2003 Elsevier Science B.V. All rts. reserv.

10983252 EMBASE No: 2001025464
Lesional sequence of acute respiratory distress syndrome: Histologic aspects
SEQUENCE LESIONNELLE DU SYNDROME DE DETRESSE RESPIRATOIRE AIGUE, ASPECTS HISTOLOGIQUES
Papazian L.; Gainnier M.; Bregeon F.
L. Papazian, Reanimation Medicale, Hopital Sainte-Marguerite, 270, boulevard de Sainte-Marguerite, 13009 Marseille France
Reanimation Urgences (REANIM. URGENCES) (France) 2000, 9/8 (621-627)
CODEN: REURF ISSN: 1164-6756
DOCUMENT TYPE: Journal ; Conference Paper
LANGUAGE: FRENCH
NUMBER OF REFERENCES: 61

2/3/27 (Item 11 from file: 73)
DIALOG(R)File 73:EMBASE
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10983251 EMBASE No: 2001025463
Chronology and 'compartmentalization' of the inflammatory response during septic shock and **adult respiratory distress** syndrome
CHRONOLOGIE ET <<COMPARTIMENTALISATION>> DE LA REPONSE INFLAMMATOIRE AU COURS DU CHOC SEPTIQUE ET DU SYNDROME DE DETRESSE RESPIRATOIRE DE L'ADULTE
Pugin J.
J. Pugin, Div. des Soins Intensifs de Medecine, Hopital Cantonal Universitaire, 24, rue Micheli-du-Crest, 1211 Geneve 14 Switzerland
AUTHOR EMAIL: pugin@cmu.unige.ch
Reanimation Urgences (REANIM. URGENCES) (France) 2000, 9/8 (613-620)
CODEN: REURF ISSN: 1164-6756
DOCUMENT TYPE: Journal ; Conference Paper
LANGUAGE: FRENCH
NUMBER OF REFERENCES: 39

2/3/28 (Item 12 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2003 Elsevier Science B.V. All rts. reserv.

07885820 EMBASE No: 1999341906
Measurement of cytokines in bronchoalveolar lavage fluid
Strieter R.M.; Miller E.J.; Kurdowska A.K.; Reid P.T.; Donnelly S.C.
S.C. Donnelly, Rayne Laboratory, University Medical School, University of Edinburgh, Edinburgh EH8 9AG United Kingdom
European Respiratory Review (EUR. RESPIR. REV.) (Denmark) 1999, 9/66 (106-112)
CODEN: EREWE ISSN: 0905-9180
DOCUMENT TYPE: Journal; Review
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 51

2/3/29 (Item 13 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2003 Elsevier Science B.V. All rts. reserv.

06810521 EMBASE No: 1997093010
Soluble **transforming growth factor**-alpha is present in

the pulmonary edema fluid of patients with acute lung injury
Chesnutt A.N.; Kheradmand F.; Folkesson H.G.; Alberts M.; Matthay M.A.
Dr. M.A. Matthay, Cardiovascular Research Institute, University of California, Box 0130, San Francisco, CA 94143-0130 United States
Chest (CHEST) (United States) 1997, 111/3 (652-656)
CODEN: CHETB ISSN: 0012-3692
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 27

2/3/30 (Item 14 from file: 73)
DIALOG(R)File 73:EMBASE

06583283 EMBASE No: 1996247900
Matrix metalloproteinases and TIMP in acute respiratory distress syndrome
Ricou B.; Nicod L.; Lacraz S.; Welgus H.G.; Suter P.M.; Dayer J.-M.
Div. Soins Intensifs Chirurgicaux, Hopital Cantonal Universitaire,1211 Geneve 14 Switzerland
American Journal of Respiratory and Critical Care Medicine (AM. J. RESPIR. CRIT. CARE MED.) (United States) 1996, 154/2 (346-352)
CODEN: AJCME ISSN: 1073-449X
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

2/3/31 (Item 15 from file: 73)
DIALOG(R)File 73:EMBASE

06530174 EMBASE No: 1996186008
Targeting the alveolar epithelium in acute lung injury: Keratinocyte growth factor and regulation of the alveolar epithelial barrier
Matuschak G.M.; Lechner A.J.
Saint Louis Univ. Health Sci. Center,St. Louis, MO United States
Critical Care Medicine (CRIT. CARE MED.) (United States) 1996, 24/6 (905-907)
CODEN: CCMDC ISSN: 0090-3493
DOCUMENT TYPE: Journal; Editorial
LANGUAGE: ENGLISH

2/3/32 (Item 16 from file: 73)
DIALOG(R)File 73:EMBASE

06519657 EMBASE No: 1996185319
The role of cytokines in human lung fibrosis
Vaillant P.; Menard O.; Vignaud J.-M.; Martinet N.; Martinet Y.
Fed. Medico-Chirurgicale Pneumologie, CHU de Nancy-Brabois, Allee du Morvan,54511 Vandoeuvre-les-Nancy France
Monaldi Archives for Chest Disease (MONALDI ARCH. CHEST DIS.) (Italy) 1996, 51/2 (145-152)
CODEN: MACDE ISSN: 1122-0643
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

2/3/33 (Item 17 from file: 73)
DIALOG(R)File 73:EMBASE

06446695 EMBASE No: 1996109847

Lung alveolar epithelial cell migration in vitro: Modulators and regulation processes
Lesur O.; Arsalane K.; Lane D.
Unite de Recherche Pulmonaire, University of Sherbrooke,Sherbrooke, Que. J1H 5N4 Canada
American Journal of Physiology - Lung Cellular and Molecular Physiology (AM. J. PHYSIOL. LUNG CELL. MOL. PHYSIOL.) (United States) 1996, 270/3 14-3 (L311-L319)
CODEN: APLPE ISSN: 1040-0605
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

2/3/34 (Item 18 from file: 73)
DIALOG(R)File 73:EMBASE

06082488 EMBASE No: 1995112975
Modifying the host response to injury: The future of trauma care
Hebert J.C.; O'Reilly M.; Bednar M.M.
Department of Surgery, University of Vermont, Medical Center Hospital of Vermont,Burlington, VT 05401 United States
Surgical Clinics of North America (SURG. CLIN. NORTH AM.) (United States) 1995, 75/2 (335-349)
CODEN: SCNAA ISSN: 0039-6109
DOCUMENT TYPE: Journal; Review
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

2/3/35 (Item 19 from file: 73)
DIALOG(R)File 73:EMBASE

05773910 EMBASE No: 1994184788
Mediators of acute and chronic pulmonary hypertension (Part 1)
Gossage J.R.; Christman B.W.
Vanderbilt University, B1308 MCN, 1161 21st Avenue South,Nashville, TN 37332-2650 United States
Seminars in Respiratory and Critical Care Medicine (SEMIN. RESPIR. CRIT. CARE MED.) (United States) 1994, 15/3 (190-198)
CODEN: SRCCE ISSN: 1069-3424
DOCUMENT TYPE: Journal; Review
LANGUAGE: ENGLISH

2/3/36 (Item 20 from file: 73)
DIALOG(R)File 73:EMBASE

05536951 EMBASE No: 1993305050
Cytokines in relation to ICU problems
Wardle E.N.
21 Common Road,North Leigh OX8 6RD United Kingdom
Clinical Intensive Care (CLIN. INTENSIVE CARE) (United Kingdom) 1993, 4/4 (183-189)
CODEN: CICAE ISSN: 0956-3075
DOCUMENT TYPE: Journal; Review
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

2/3/37 (Item 21 from file: 73)
DIALOG(R)File 73:EMBASE

05532063 EMBASE No: 1993300162
Cytokines in lung and airways fibrosis
Jordana M.; Ohno I.; Xing Z.; Gauldie J.
Department of Pathology, McMaster University, 1200 Main Street West,Hamilton, Ont. L8N 3Z5 Canada
Regional Immunology (REG. IMMUNOL.) (United States) 1993, 5/3-4 (201-206)
CODEN: REGIE ISSN: 0896-0623
DOCUMENT TYPE: Journal; Review
LANGUAGE: ENGLISH

2/3/38 (Item 22 from file: 73)
DIALOG(R)File 73:EMBASE
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05453265 EMBASE No: 1993221364
Cytokines and the lung
ZYTOKINE UND DIE LUNGE
Menard O.; Martinet N.; Martinet Y.
Clinique Pneumologique, Hopital de Brabois, CHU Nancy, Rue du Morvan,Vandeouvre Les Nancy France
Pneumologie (PNEUMOLOGIE) (Germany) 1993, 47/7 (427-438)
CODEN: PNEME ISSN: 0934-8387
DOCUMENT TYPE: Journal; Review
LANGUAGE: GERMAN

2/3/39 (Item 23 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2003 Elsevier Science B.V. All rts. reserv.

04008048 EMBASE No: 1989177044
Pathogenesis of pulmonary fibrosis: the role of cytokines
LA GENESE DES FIBROSES PULMONAIRES: PLACE DES CYTOKINES
Martinet Y.
Service de Pneumologie, CHR, 54037 Nancy Cedex France
Reanimation Soins Intensifs Medecine d'Urgence (REANIM. SOINS INTENSIFS MED. URGENCE) (France) 1989, 5/4 (265-270)
CODEN: RSIUE ISSN: 0765-5290
DOCUMENT TYPE: Journal
LANGUAGE: FRENCH SUMMARY LANGUAGE: ENGLISH

2/3/40 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

15074455 22735455 PMID: 12851645
Significant Involvement of CCL2 (MCP-1) in Inflammatory Disorders of the Lung.
Rose C Edward; Sung Sung-Sang J; Fu Shu Man
Division of Pulmunory and Critical Care Medicine and the Division of Rheumatology and Immunology, University of Virginia School of Medicine, Charlottesville, VA, USA.
Microcirculation (New York, N.Y. - 1994) (United States) Jul 2003, 10 (3-4) p273-88, ISSN 1073-9688 Journal Code: 9434935
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: In Process

2/3/41 (Item 2 from file: 155)

DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

10435831 96242491 PMID: 8678788
Cytokines in human lung fibrosis.
Martinet Y; Menard O; Vaillant P; Vignaud J M; Martinet N
INSERM U 14, Nancy-Vandoeuvre.
Archives of toxicology. Supplement. Archiv fur Toxikologie. Supplement (GERMANY) 1996, 18 p127-39, ISSN 0171-9750 Journal Code: 7802567
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

2/3/42 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

09615892 21401555 PMID: 11510779
Vascular endothelial growth factor synthesis in the acute phase of experimental and clinical lung injury.
Maitre B; Boussat S; Jean D; Gouge M; Brochard L; Housset B; Adnot S; Delclaux C
INSERM Unite 492, Dept de Physiologie, Hopital Henri-Mondor, Creteil, France.
European respiratory journal - official journal of the European Society for Clinical Respiratory Physiology (Denmark) Jul 2001, 18 (1) p100-6, ISSN 0903-1936 Journal Code: 8803460
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

2/3/43 (Item 1 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)
(c) 2003 American Chemical Society. All rts. reserv.

130020575 CA: 130(3)20575x PATENT
Method for treating ischemia using polypeptides with fibronectin activity
INVENTOR(AUTHOR): Furcht, Leo T.; McCarthy, James B.; Wahl, Sharon M.; Allen, Janice B.; Billups, Kevin L.; Everett, Jeffrey E.
LOCATION: USA
PATENT: United States ; US 5840691 A DATE: 19981124
APPLICATION: US 480133 (19950607) *US 990296 (19921210) *US 139903 (19931021)
PAGES: 35 pp., Cont.-in-part of U.S. Ser. No. 139,903, abandoned.
CODEN: USXXAM LANGUAGE: English CLASS: 814012000; A61K-038/04A; A61K-038/08B; A61K-038/10B; A61K-038/16B

2/3/44 (Item 2 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)
(c) 2003 American Chemical Society. All rts. reserv.

126304819 CA: 126(23)304819x JOURNAL
Hemorrhage increases cytokine expression in lung mononuclear cells in mice. Involvement of catecholamines in nuclear factor .kappa.B regulation and cytokine expression
AUTHOR(S): Le Tuizo, Yves; Shenkar, Robert; Kaneko, Debra; Moine, Pierre; Fantuzzi, Giamila; Dinarello, Charles A.; Abraham, Edward
LOCATION: Division of Pulmonary Sciences and Critical Care Medicine, University of Colorado Health Sciences Center, Denver, CO, 80262, USA

JOURNAL: J. Clin. Invest. DATE: 1997 VOLUME: 99 NUMBER: 7 PAGES: 1516-1524 CODEN: JCINAO ISSN: 0021-9738 LANGUAGE: English PUBLISHER: Rockefeller University Press

2/3/45 (Item 3 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

126017804 CA: 126(2)17804h PATENT
Human antibodies derived from immunized xenomice
INVENTOR(AUTHOR): Kucherlapati, Raju; Jakobovits, Aya; Klapholz, Sue; Brenner, Daniel G.; Capon, Daniel J.
LOCATION: USA
ASSIGNEE: Cell Genesys, Inc.
PATENT: PCT International ; WO 9634096 A1 DATE: 19961031
APPLICATION: WO 95US5500 (19950428)
PAGES: 64 pp. CODEN: PIXXD2 LANGUAGE: English CLASS: C12N-015/00A
DESIGNATED COUNTRIES: AU; CA; FI; HU; JP; KR; NO; NZ
DESIGNATED REGIONAL: AT; BE; CH; DE; DK; ES; FR; GB; GR; IE; IT; LU; MC; NL; PT; SE

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Items   Description
S1         29   (LACI OR LIPOPROTEIN(W)ASSOCIATED(W)COAGULATION) AND COAGU-
             LATION(W)(DISORDER? OR DISEASE?)
S2         28   RD S1 (unique items)
S3          0   S2 AND PY<1988
S4        383   (LACI OR LIPOPROTEIN(W)ASSOCIATED(W)COAGULATION) AND (DISO-
             RDER? OR DISEASE?)
S5          2   S4 AND PY<1989
S6          1   RD S5 (unique items)
S7       4094   (LACI OR LIPOPROTEIN(W)ASSOCIATED(W)COAGULATION)
S8       1030   S7 AND COAGULATION
S9         22   S8 AND PY<1989
S10        11   RD S9 (unique items)
S11      3869   (FACTOR(W)XA OR FACTOR(W)VIIA OR TISSUE(W)FACTOR) AND COAG-
             ULATION  AND (DISORDER? OR DISEASE?)
S12       448   (FACTOR(W)XA OR FACTOR(W)VIIA OR TISSUE(W)FACTOR)(10N)(INH-
             IBIT? OR SUPPRESS?) AND COAGULATION AND (TREAT? OR PREVENT? OR
              THERAP?)(10N)(DISORDER? OR DISEASE?)
S13        10   S12 AND PY<1989
S14         7   RD S13 (unique items)
?
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Gamb I, Phillip

To: STIC-ILL
Subject: laci two

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please provide the following references to

phillip gambel
art unit 1644
308-3997

1644 mailbox 9e12

14/7/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

02121116 BIOSIS NO.: 000063036112
PENICILLIN INDUCED COAGULATION DISORDER
AUTHOR: ANDRASSY K; SCHERZ M; RITZ E; WALTER E; HASPER B; STORCH H; VOEMEL W
JOURNAL: LANCET 2 (7994). 1976 1039-1041. 1976
FULL JOURNAL NAME: Lancet
CODEN: LANCA
RECORD TYPE: Abstract

ABSTRACT: A coagulation disorder was seen after penicillin G administration (10 million units/day) in uremic patients and after high-dose penicillin G (40 million units/day) in patients with a normal glomerular filtration rate (5 patients after cardiac surgery). This disorder was characterized by prolongation of bleeding time, appearing immediately after penicillin G administration and persisting until 4 days after withdrawal of therapy; disturbance of collagen-induced and ristocetin-induced platelet aggregation; increase of antithrombin-III activity; and inhibition of factor-Xa activity. The inhibition of factor-Xa activity corresponded to that seen after low-dose heparin prophylaxis. The clinically latent coagulation disorder, when superimposed upon pre-existing coagulation abnormalities (uremia, treatment with anticoagulants) may cause severe bleeding, as observed in 1 patient with acute renal failure on hemodialysis.

14/7/2 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

03813081 EMBASE No: 1988262521
Factor VIII-bypassing activity of bovine tissue factor using the canine hemophilic model
O'Brien D.P.; Giles A.R.; Tate K.M.; Vehar G.A.
Department of Cardiovascular Research, Genetech, Inc., South San Francisco, CA 94080 United States
Journal of Clinical Investigation (J. CLIN. INVEST.) (United States) 1988, 82/1 (206-211)
CODEN: JCINA ISSN: 0021-9738

DOCUMENT TYPE: Journal
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

The bleeding disorder of hemophilia A currently treated by replacement therapy of the missing coagulation factor, factor VII, is frequently complicated by the development of neutralizing antibodies. The therapeutic potential of attenuated forms of the lipid-associated glycoprotein tissue factor, a known inhibitor of coagulation, was investigated as a factor VIII-bypassing activity. The protein moiety of tissue factor (Apo-TF) was partially purified and exhibited minimal procoagulant activity before relipidation in vitro. In pilot studies, Apo-TF injection into rabbits previously anticoagulated with an antibody to factor VIII was found to have a procoagulant effect. The efficacy of the material was further demonstrated when injection of Apo-TF in hemophilic dogs resulted in a normalization of the cuticle bleeding time. Little or no change in the blood parameters associated with disseminated intravascular coagulation was observed at lower doses, although mild to moderate effects were seen at higher doses. These data suggest a novel role for Apo-TF preparations as a potential therapeutic agent for hemophilics with antibodies to factor VIII once the potential thrombogenicity of such materials is evaluated.

14/7/3 (Item 2 from file: 73)
DIALOG(R)File 73:EMBASE

02350096 EMBASE No: 1983229100
Molecular markers of hemostatic disorders: Implications in the diagnosis and therapeutic management of thrombotic and bleeding disorders
Fareed J.; Bick R.L.; Squillaci G.; et al.
Loyola Univ. Med. Cent., Maywood, IL 60153 United States
Clinical Chemistry (CLIN. CHEM.) (United States) 1983, 29/9 (1641-1658)
CODEN: CLCHA
DOCUMENT TYPE: Journal
LANGUAGE: ENGLISH

With current technological advances, it is now possible to measure in <50 muL of plasma picomolar amounts of circulating products of platelet activation, products of protease activation related to coagulation and fibrinolytic pathways, and prostaglandin metabolites formed during a physiologic or pathologic process. Most of these markers, which circulate in blood in nanogram or picogram amounts per milliliter during or after pathologic activation, provide pertinent information on the status of a patient in terms of specificity and early detection, and will be of crucial value in the diagnosis of hemostatic defects and the management of newer antithrombotic drugs that cannot be monitored by currently available assays. Currently, sup 1sup 2sup 5I- and sup 3H-based simple radioimmunoassays are available for platelet factor 4, beta-thromboglobulin, fibrinopeptide A, Bbeta 15-42 related peptides, thromboxane Binf 2, and the prostaglandins 6-keto-PGF(inf 1alpha) and PGEinf 2. Nonisotopic methods such as enzyme-linked immunosorbent assays and fluoroimmunoassays are being developed. Serotonin and ADP, products of platelet activation, are measurable by liquid-chromatographic, immunoenzymatic, and spectrophotofluorometric methods. Analytical methods for fibrin split products (fragments D and E) and serine protease inhibitor complexes such as thrombin-antithrombin-III, factor Xa-antithrombin-III, and kallikrein-Cinf 1-esterase are also being developed. We have evaluated all of these methods and found them to be very

sensitive to those components of endogenous activation of the hemostatic system listed above.

14/7/4 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

04998841 85305963 PMID: 3929705
Coronary bypass surgery in patients with circulating lupus anticoagulant.
Kelly J P; Thomas L; Moulder P V; Webb W R
Annals of thoracic surgery (UNITED STATES) Sep 1985, 40 (3) p261-3, ISSN 0003-4975 Journal Code: 15030100R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Clinical and laboratory experience with circulating lupus anticoagulant in 3 patients undergoing coronary artery bypass procedures is reported. This circulatory anticoagulant inhibits activation of prothrombin by the prothrombin activator complex (factor Xa, factor V, and phospholipid). The presence of lupus anticoagulant was initially detected because of a prolonged activated partial thromboplastin time and a normal or mildly prolonged prothrombin time. The 3 patients underwent uncomplicated coronary artery bypass grafting and experienced no abnormal bleeding postoperatively. The lupus anticoagulant is a rare cause of bleeding after open-heart surgery. It appears to be a problem only when an additional coagulation defect is present.
Record Date Created: 19851001
Record Date Completed: 19851001

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14/7/5 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

04938601 85245383 PMID: 4012669
Comparative effects of heparin and PK 10169, a low molecular weight fraction, in a canine model of arterial thrombosis.
Mestre M; Clairefond P; Mardiguian J; Trillou M; Le Fur G; Uzan A
Thrombosis research (UNITED STATES) May 15 1985, 38 (4) p389-99, ISSN 0049-3848 Journal Code: 0326377
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The comparative properties of heparin and PK 10169, a low molecular weight fraction, were studied using an antithrombotic test in anaesthetized dogs. The antithrombotic properties of the two compounds were evaluated by measuring inhibition of thrombus formation following transluminar stimulation of coronary artery with anodal current and by measuring anticoagulant properties, anti Xa and anti IIa activities. The results show that PK 10169 displayed significant antithrombotic activities above 0.625 mg/kg and was equipotent at 2.5 mg/kg s.c. with heparin 10 mg/kg s.c. No correlation could be observed between antithrombotic/anti Xa ratio of both compounds. Moreover it was shown that, unlike heparin, PK 10169 s.c. was devoid of obvious anticoagulant properties and induced a negligible anti IIa activity contrasting with a high anti Xa level. A similar dissociation between anti Xa and anti IIa activities was observed following i.v. administration of 2.5 mg/kg of PK 10169 but not with heparin. This low

molecular weight heparin fraction might thus be regarded as a potential arterial antithrombotic agent devoid of appreciable anticoagulant effect.
Record Date Created: 19850812
Record Date Completed: 19850812

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14/7/7 (Item 1 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

104202975 CA: 104(23)202975y JOURNAL
Isolation and characterization of human antithrombin III
AUTHOR(S): Trobisch, H.; Wuest, T.; Rustige, H.
LOCATION: Inst. Laboratoriumsmed., D-4100, Duisburg, Fed. Rep. Ger.
JOURNAL: Behring Inst. Mitt. DATE: 1986 VOLUME: 79, PAGES: 177-90
CODEN: BHIMA2 ISSN: 0301-0457 LANGUAGE: German
SECTION:
CA107003 Enzymes
CA101XXX Pharmacology
IDENTIFIERS: antithrombin III isolation property, blood coagulation disorder antithrombin III
DESCRIPTORS:
Blood coagulation,disorder...
antithrombin III in treatment, in humans
Blood plasma...
antithrombin III of, of human, isolation and characterization of
Amino acids,biological studies...
of antithrombin III, of human
Michaelis constant...
of blood-coagulation factor Xa, antithrombin III of human and heparin effect on
Kinetics,enzymic...
of inhibition, of blood-coagulation factor Xa by human antithrombin III, heparin effect on
CAS REGISTRY NUMBERS:
9005-49-6 biological studies, antithrombin III of human interaction with, in inhibition of blood-coagulation factor Xa
9000-94-6 III, of human plasma, isolation and characterization of
9002-05-5 inhibition of, by human antithrombin III, kinetics of, heparin effect on
60457-00-3 reaction of, with blood-coagulation factor Xa, kinetics of, antithrombin III of human and heparin effect on